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PREFACE.

THIS little book has been designed by the writer to serve the needs of the medical student preparing for examination, and for the practitioner of medicine who desires to acquaint himself with the principal facts of the rapidly growing science of bacteriology.

An effort has been made to reduce the subject matter to as concrete form as possible.

While the literature of the subject of immunity is as vast almost as the rest of bacteriology, yet it is hoped that the chapter in this book on immunity gives, in outline, the essential accepted teachings on the subject.

Minute details of cultures and technic are not given, they must be sought for in books on descriptive bacteriology.

The author has freely drawn from books by Muir and Ritchie, Williams, McFarland, Lehmann and Neumann, Ricketts, and Bosanquet. Many of the illustrations are from Kolle and Wassermann's atlas, Williams, McFarland, Tyson's practice and Abbott.

The writer is much indebted to Mr. C. V. Brownlow, with the firm of P. Blakiston's Son & Co., for his invaluable aid.

ROBERT L. PITFIELD.

Germantown, Philadelphia, Pa.

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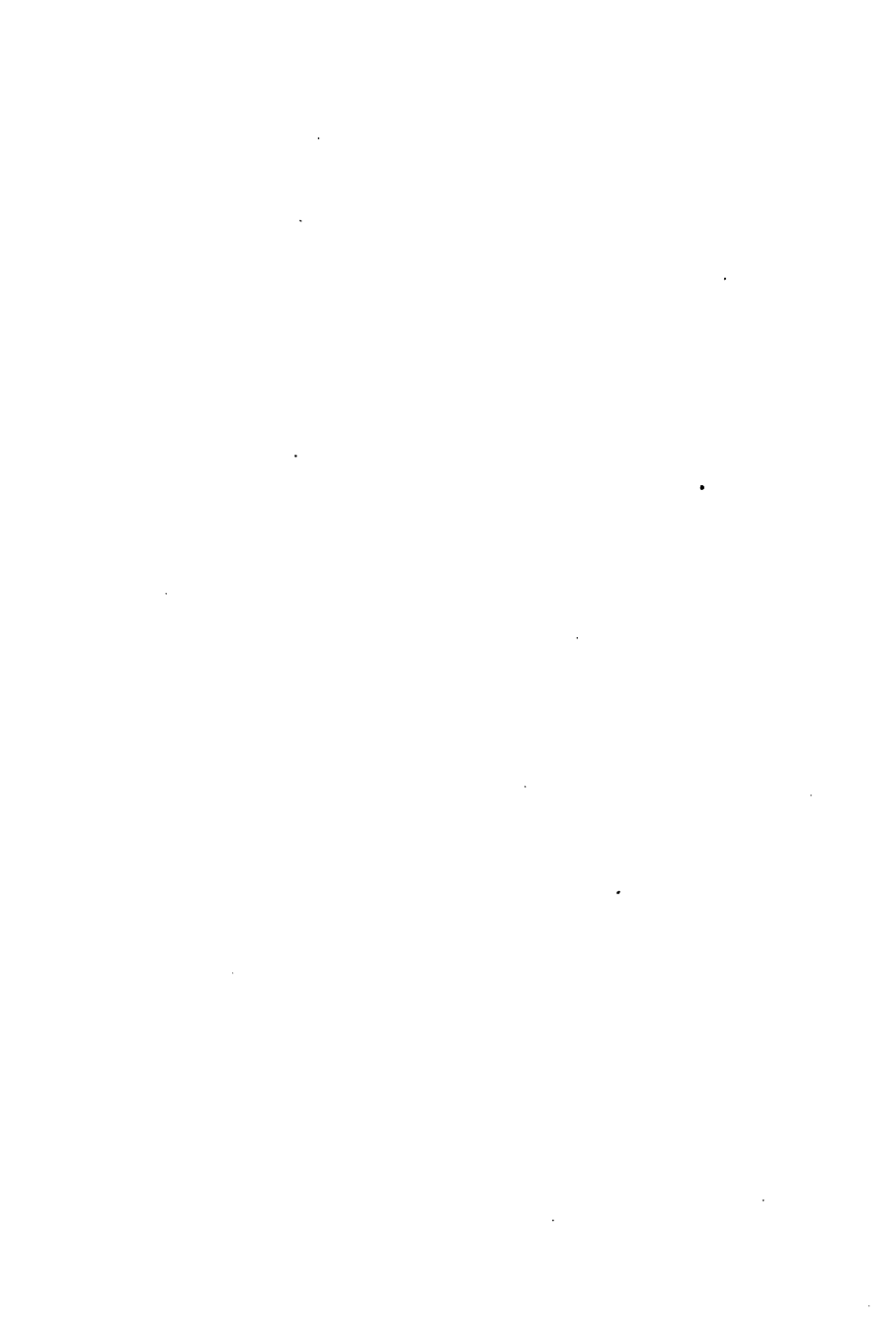
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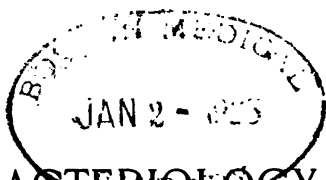
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COMPEND OF BACTERIOLOGY.

CHAPTER I.

THE CLASSIFICATION, MORPHOLOGY, AND THE BIOLOGY OF BACTERIA.

BACTERIA.—(fission fungi or *schizomycetes*) may be defined as very minute, unicellular vegetable organisms, almost always devoid of chlorophyll, and generally unbranched, that reproduce themselves asexually by means of direct division or fission, spores, or gonidia. They are allied closely on the one hand to the higher fungi, such as the moulds, and on the other to the algæ. Many forms in one phase of development closely resemble members of other groups, and it has always been difficult to classify them. Various botanical classifications have been employed by different bacteriologists. The following one is based somewhat upon *Migula's*, and that adopted by *Lehmann and Neumann*, which was compiled from the systems of *Flügge, Fischer, Löffler, and Migula*.

CLASSIFICATION.—Bacteria may be conveniently divided into six families, according to their morphology or shape.

I. COCCACEÆ.—Spherical bacteria in which no diameter is greater than any other. To multiply, the cell divides into halves, quarters, or eighths, each of which grow again into perfect spheres. Endospores and flagella are very rare. (*Lehmann and Neumann*.)

(a) **Streptococcus.**—Cells that divide in one direction only and grow in chains.

(b) **Micrococcus.**—Cells that divide in two directions, or

irregularly; with this group **staphylococcus** may be classed. Also tetrads, which form into fours by division in two directions.

- (c) **Sarcina**.—Cells that divide in three directions so that balelike packages, or blocks of eight are formed. At least one variety (*Sarcina Agilis*) is motile, having flagella.

II. BACTERIACEÆ.—Rod bacteria are straight or slightly curved. Each cell is from two to six times as long as broad. Division takes place in one direction only, and at right angles to the long axis. Spores may be produced and may not. They may have flagella, or may not.

- (a) **Bacterium**. Have no endospores.
(b) **Bacillus**. Have endospores, and often grow in long threads.
(c) **Pseudomonas**. Have endospores very rarely.

III. SPIRILLACEÆ.—Spiral bacteria. Unicellular, more or less elongated. Cells are sometimes united in short chains. Generally very motile. Spores are known in two varieties only.

- (a) **Vibrio**. Cells that are rigidly bent like a comma, and have always one, occasionally two polar flagella.
(b) **Spirillum**. Are long and spiral, like a corkscrew, are rigid, and have a bunch of polar flagella.
(c) **Spirochæte**. Cells with long flexible spiral threads, without flagella. They move by means of an undulating membrane.

IV. MYCOBACTERIACEÆ.—Cells with short or long filaments, which are often cylindrical, clavate (club shaped) or irregular in outline, and display true or false branching. Spores are not formed, but gonidia are. They have no flagella, and division takes place at right angles to the long axis. There is no surrounding sheath as in the next family (V).

- (a) **Mycobacterium.** Cells are short cylindrical rods, sometimes wedge-like, bent, or Y shaped: long and filamentous. They exhibit true branching, and perhaps produce coccoid elements and gonidia, but no flagella. The **Corynebacterium** of *Lehmann and Neumann* belongs to this group. Many are *acid fast*.
- (b) **Actinomyces** (*ray fungus*) are long mycelial threads, that radiate in indian-club, or loop-like forms, with true branching and delicate sheaths, devoid of gonidia and flagella. Growth coherent, mould-like and dry. Often powdery on the surface in culture media. Not acid proof or acid fast, and frequently emit a musty odor.

V. **CHLAMYDOBACTERIACEÆ.—Sheathed bacteria.** Cells are characterized by an enveloping sheath about branched and unbranched threads. Division takes place at right angles to the long axis of the cells.

- (a) **Cladothrix** are distinguished by false dichotomous branching. Multiplication is affected by separation of whole branches, and by swarm spores or motile gonidia having flagella.
- (b) **Crenothrix.** Filaments are fixed to a nutrient base. Are usually thinner at the base than at the apex, formed of unbranched threads that divide in three directions of space, and produce in the end two kinds of gonidia.
- (c) **Phragmidiothrix.** Cells are first united into unbranched threads by means of delicate sheaths, branching threads are then formed. Division takes place in three directions of space, producing sarcina-like groups of gonidia, which, when free, are spherical.
- (d) **Thiothrix.** Are unbranched cells, sheathed, without flagella, divided only in one direction, and contain sulphur granules.

VI. BEGGIATOACEÆ. Cells united to form threads that are not sheathed: have scarcely visible septa; divide in one direction, and motile only by an undulating membrane, not by flagella.

(a) **Beggiatoa.** Cells containing sulphur granules.

Bacteria may furthermore be classified according to their biological characteristics, which may be wonderfully different. The ultimate differentiation of one species from another depends not only on the morphology, which may be precisely similar, but on its biological behavior in culture media and in the tissues of animals under



FIG. 1.—Large and very large streptococci. (Kolle and Wassermann.)

identical conditions. Again, different individuals of a given species may vary extraordinarily one from another in form and size, yet the chemical behavior is invariably the same. Hence it is only by observation of the development of bacteria in culture media, and the reactions produced in it, and in the bodies of experiment animals, that we can identify them positively from others of a foreign species. No

bacteriologist is able by a simple microscopical examination of a given bacterium, to identify it absolutely at all times.

Bacteria that are globular in form are called *cocci*.

Cocci that divide in one direction of space and grow in chains are called *streptococci*. (Fig. 1.)

Cocci that divide irregularly and form pairs or fours, or irregular groups, are called *micrococci*. Those of this class that form pairs are frequently called *diplococci*. When they form fours by division in two directions, they are called *tetrads*. But when they divide



FIG. 2.—Staphylococci. Streptococci. Diplococci. Tetrads. Sarcinae. (Williams.)

irregularly and form masses resembling bunches of grapes, they are spoken of as *staphylococci*. (Fig. 2.)

Cocci that divide in three directions are called *sarcinae*. One single coccus, by division in three directions, forms cubes of eight or more, each of which becomes globular and equal in size to the parent.

Motile micrococci are those that divide in two directions of space, and have flagella. They are known as *planococci*.

Micrococci that divide in *three* directions, and are motile, are called *planosarcina*. (Fig. 3.)

Bacteria that resemble straight rods are called *bacilli*. These may be short and thick, or long and thread-like; are never curved, but may be slightly bent.

Bacilli may grow singly or in chains; may be flagellated; contain spores and gonidia; or, may be devoid of flagella.

Members of the spirillaceæ that resemble a curved rod, or are

comma shaped, are known as *vibrios*. (Fig. 4.) Those of the same family that resemble a cork-screw, are called *spirilla*. (Fig. 5.) When they are like long spiral threads they are called *spirochæte*.

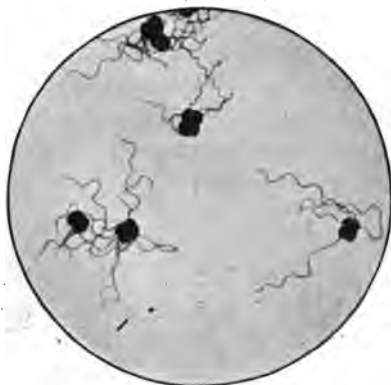


FIG. 3.—*Planosarcina ureæ*, showing very long flagella.
(Kolle and Wassermann.)

Any of these different members of the family of spirillace may grow in chains.

In clinical medicine it is common to speak of the streptococcus



FIG. 4—Cholera vibrios.
(Greene's Medical Diagnosis.)



FIG. 5.—*Spirillum* relapsing fever.
(Greene's Medical Diagnosis.)

lanceolatus as the pneumococcus. As the organism appears in the *diseased lung*, or in the *sputum*, one diameter of the coccus is invariably longer than another, and the rule of equal diameters cannot be

applied to it. But in *culture media*, the organism resembles a true coccus, being perfectly globular and growing in chains. It is then called the *streptococcus lanceolatus*. It is common also to speak of members of the family of mycobacteriaceæ as bacilli, as they are more commonly met with in this form in clinical examinations, and in cultures. Hence, we frequently hear of the bacillus of tuberculosis, and not the mycobacterium tuberculosis.

Among the higher bacteria, the differentiation of those belonging to the sheathed group, or chlamydobacteriaceæ, is difficult, as it depends largely upon the formation of the false branching and the gonidia. When bacteria exhibit many, or various forms, in the same culture, as does the typhoid bacillus, we speak of it as *pleomorphic*, or pleomorphism. To elucidate: Man is pleomorphic, because among adult individuals some are tall, or short, fat or thin.

It is common to speak of bacteria that differ from the adult forms, by reason of age and growth, as *involution* forms. To explain:—Infants, children, and those past puberty, but who have not yet become adults, are in a state of involution, or development.

To measure bacteria, we use the thousandth part of a millimeter, called the micromillimeter, or micron, as the unit. The Greek letter μ is the symbol for this unit. A micron is about $\frac{1}{25000}$ of an inch, yet a bacterium one μ long, and a half μ in width, is very large in comparison to some things that scientists measure, such as the thickness of oil films, soap bubbles, or light-wave lengths, in which the unit is a micro-micron, and is symbolized by $\mu\mu$. The shortest light-wave lengths are about 400 $\mu\mu$, or .4 μ , while Chromatic threads in cells of bacteria are often 100 $\mu\mu$ in width. Then again there are many things smaller than these threads. The thinnest part of a bursting soap bubble is but 7. $\mu\mu$ in thickness. There are certain infectious agents that are submicroscopic; that is, invisible even by the aid of Siedentopf's ultraviolet microscope, which shows objects smaller by half a light-wave length (.2 $\mu\mu$).

The structure of the bacterial cell is very simple. It consists of (1) a central nuclear body which can be stained like the nuclei of

other vegetable and animal cells, with nuclear or basic stains, such as hæmatoxylin, or methylene-blue.

(2) A cytoplasm, or protoplasmic substance generally very thin.

(3) A capsule, more or less thick, that stains with difficulty. (Fig. 6.)



FIG. 6.—Diplococci showing capsules. (Greene's Medical Diagnosis.)

In the nucleus we often see metachromatic bodies, called the *Babes-Ernst* granules, and unstained spaces called *vacuoles*, both of which are common to many bacteria.

In some bacteria the capsule is very thick and quite characteristic, often mucilaginous. It is generally the first portion of the cell to be attacked by certain specific substances (ferment) found in the blood of immunized animals, called *bacteriolysins* and *agglutinins*. Where

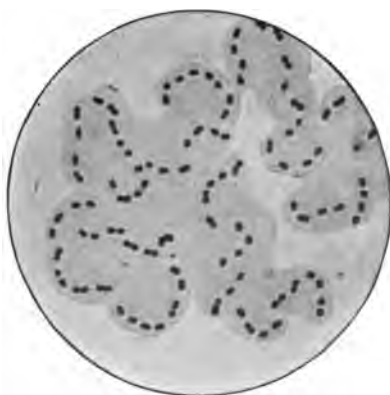


FIG. 7.—Zoöglia formation. (*Leuconostoc*.) (Kolle and Wassermann.)

great masses of bacteria are clumped in excessive mucilaginous material we speak of this condition as zoöglia. (Fig. 7.)

We sometimes find, as a prolongation of the capsule, filamentous

organs of locomotion known as flagella. Not all bacteria possess these, but those that do, are called *trichobacteria*. Those that have not flagella are called gymnobacteria. Trichobacteria are classified according to the number and location of the flagella. When they have one flagellum we call them *monotrichous* bacteria, and *amphitrichous* when there are two flagella, one at each pole. (Fig. 8.) When

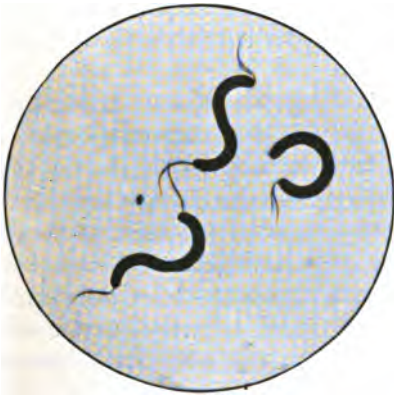


FIG. 8.—*Spirillum undula* with polar flagella. (Kolle and Wassermann.)



FIG. 9.—*Bacillus prolius vulgaris*, showing peritrichous flagella. (Kolle and Wassermann.)

the cell is surrounded by flagella, it is known as *peritrichous* bacterium, and *lophotrichous* when the flagella are arranged in tufts of two or more. The tetanus bacillus is an example of a peritrichous organism, while the bacillus of green pus is called monotrichous, because of its single flagellum. (Fig. 9.)

Flagella are not pseudopods, but distinct organs of locomotion.

In certain bacteria of the Beggiatoa, locomotion is accomplished by a peculiar amoeboid motion, or by an undulating membrane. On looking at bacteria known to have no powers of voluntary motion, they are seen to tremble or move slightly. Suspensions of india-ink in water are seen to do the same thing, as are other inanimate suspensions. This molecular movement is known as the Brownian motion. There are bacteria that are considered non-motile, on which it is possible to demonstrate flagella. By ordinary staining methods, and in preparations of living bacteria known to be flagellated, these organs of locomotion cannot be seen, as a rule. Occasionally, however, one may be seen under either condition. Generally, strong solutions of aniline dyes, to which powerful mordants have been added, are necessary to stain the capsule of bacteria and the attached flagella.

Flagella may have other functions than locomotion. It is possible that they may serve as organs for the absorption of nourishment from the surrounding media. The presence of very long or very numerous flagella does not necessarily presage very active motion. At times, under certain conditions, an organism ordinarily motile and flagellated will appear immobile and non-flagellated (*Lehmann and Zierler*), but this is rare. Certain flagella have in their continuity little round granules, or bodies, which apparently have nothing to do with the functions of locomotion, but may have something to do with the nutrition of the cell.

REPRODUCTION.—The process of cell division is the commonest way by which bacteria multiply. Hence the name of fission fungi. The other ways of reproduction are by means of bodies called *spores*, and by other chromophile granules called *gonidia*.

The process of cell division or binary division is very simple, and may be a matter of forty minutes, or as long as six hours. Division is almost always across the cell in the direction of the short axis, though it may in some bacteria be in a direction parallel to the long axis, but this is uncommon.

By means of the hanging drop or the block culture method, on

an inverted cover-glass, the process may be observed easily. The phenomena of division begin by an elongation of the cell, soon followed by an invagination of the cell on both sides, at an equatorial point. The process begins to be apparent in the cell and extends inward.

Division may occur in one, two, or three directions, or planes.

By cell division bacteria multiply by geometrical progression. One cell at the end of an hour becomes two, and at the end of a second hour these two become four; at the end of another hour these four become eight; after twenty-four hours they may number many millions.

It is well that the food supply soon gives out and that the products of bacterial metabolism, such as acids and ferments, inhibit their growth. By this rapid bacterial multiplication, carcasses of animals are disintegrated and the higher nitrogenous compounds are reduced to simple gases that are quickly dissipated in the air.

SPORULATION is a second mode of reproduction.

Spores (endospores) are highly resistant resting forms of bacteria. They are surrounded by a thick capsule, which enables them to resist chemicals, heat and drying.

It is held by some that sporulation corresponds to the flowering of the higher plants, and that they are produced under the most favorable vital conditions. The other view is, that spores are produced under stress of circumstances, when certain agencies or conditions, such as absence of food, drying, and heat, threaten the extinguishment of the organism. Spores are bright, shining, oval, or round bodies, which do not take aniline dyes readily, and which, when they are stained, retain the color more tenaciously than the adult cells. They resist heat, often withstanding a temperature of 150° C. dry heat for an hour. Steam under pressure at a temperature of 150° C. will invariably kill them after a short exposure.

Spores are situated either in the ends of the adult organism (polar) or in the middle (equatorial), and the spore is discharged (sporulation) either from the end or through the side.

The spore is developed in the bacterial cell as follows:—If the organism is a mobile one it becomes quiet before sporulation, during which the flagella are retained. The diameter becomes greater in one portion of the cell, and dust-like particles appear, then a bright spot; a capsule then forms, the spore escapes, and the parent cell dies.

Certain spore bearing bacteria grown for a week at 42° C. lose the power to form spores, likewise their progeny. As a rule the anthrax bacillus does not form spores in the bodies of animals. Free oxygen

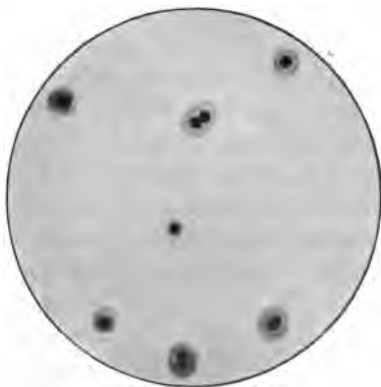


FIG. 10.—Pest bacilli showing capsules. (Kolle and Wassermann.)

is required for sporulation by some bacteria. One spore only is produced by an adult cell. Some forms of bacteria can be differentiated from each other only by the way in which they sporulate, whether from the poles or the equator.

The families of **Bacteriaceæ** and **Spirillaceæ** are the prominent spore producers. Certain round bodies found in bacteria of low thermal death-point, are called by Heuppe *arthrospores*. It is believed that they are without significance. A high thermal death-point in bacteria indicates that the organism produces spores.

Spores resist chemicals for a long period, and withstand drying, even in lime plaster, for years. It is believed that the thick capsule enables them to resist these deleterious agents.

Sporulation is more apt to occur under poor nutritive conditions.

The anthrax bacillus thrives at 13°C . but cannot sporulate below 18°C . Anthrax spores have been known to resist the anti-septic action of a 5 percent carbolic acid solution for forty days.

Gonidia (*Babes-Ernst granules*, or *polar bodies*) are found in certain bacteria (*Mycobacteriacæ*, etc.) after staining with special basic stains. In the complex forms of bacteria, they evidently have an important rôle in reproduction. The presence of such bodies in the poles of diphtheria bacilli facilitates the recognition of these organisms. (Fig. 10.)

Capsules. Certain well known pathogenic bacteria have thick well marked capsules. The *pneumococcus*, *pneumobacillus*, and *bacillus aerogenes capsulatus*, are well known examples of such capsulated organisms. The capsule is not always constant. It often disappears when the organism is grown in culture media. (Fig. 11.)



FIG. 11.—Bacilli showing capsules. (Greene's Medical Diagnosis.)

THE CHEMICAL COMPOSITION OF BACTERIA.

Bodies of bacteria contain water, salts, certain albumins, and bodies that may be extracted with ether. Among the latter are lecithin, cholesterin, and triolein. In the tubercle bacillus, fatty acids and wax have been found. In others, xanthin bases, cellulose, starch, chitin, iron salts, and sulphur grains have been discovered. The essential albumin of the cell-body is highly nitrogenous and is called *mycoprotein*. The salts in the ash are mostly composed of various phosphates. Intracellular toxins in combination with the cytoplasm are found in certain groups of bacteria, e.g., *B. typhoid*.

BIOLOGICAL CONDITIONS.

Bacteria are arbitrarily classed either as *parasites*, or *saprophytes*. They may be so dependent upon the tissues of the infected organism as to be a strict parasite and incapable of growth under any other condition (*Mycobact. Lepre*), or they may be capable of life on artificial culture media (tubercle bacillus), or of life in the body, on culture media, or in the soil (*B. tetanus*).

The bacteria of the soil, water, air, etc., that are incapable of successful life in the body tissues are called saprophytes.

Certain biological conditions are essential for the growth of bacteria:—water, carbon, nitrogen, and salts are necessary. For certain parasitic bacteria, highly complex substances are indispensable:—meat albumins, peptones, milk, egg albumin, blood serum, and sugars are the ingredients of various culture media.

The chemical reaction of such media is important: it should either be faintly acid or alkaline. The greatest number of water bacteria grow in media that are slightly acid, while diphtheria produces its strongest toxins and grows best in alkaline media.

All bacteria require for their growth either free oxygen, as in air, or combined oxygen, as in albumin, water, etc. Those that only grow when deprived of free oxygen are known as *obligate anaerobes*, while those that require the presence of oxygen are called *obligate aerobes*. Those that grow under either conditions are named *facultative anaerobes*. Free oxygen is needed for spore formation by certain bacteria.

Nutrient is most important for the growth of bacteria, nitrogenous compounds (albumins) particularly being required. Simple aquatic forms of bacteria can live and grow in distilled water. The addition of the various sugars is of advantage in the cultivation of many bacteria, and glycerine for the growth of some members of the *mycobacteriaceæ*.

The **temperature** of the medium in which various bacteria grow is most important. Bacterial growth is possible between 0° C. and 70° C., some varieties thrive at the one extreme, and others at the other.

Psychrophilic bacteria, are those that grow at 15°C. , with a maximum of 30°C. and a minimum of 0°C. Water bacteria of the polar seas belong to this group.

Mesophilic grow best at 37°C. —the temperature of the body—and thrive from 10°C. (minimum) to 45°C. (maximum). All pathogenic bacteria belong to this group.

Thermophilic (min. temp. 40°C. , max. $60\text{--}70^{\circ}\text{C.}$) are most prolific at $50\text{--}55^{\circ}\text{C.}$ To this class belong bacteria of the soil. All of this class are spore bearing.

Darkness favors bacterial growth.

Association of different kinds of bacteria is of some importance in their growth and welfare. When thus associated, they sometimes benefit each other. Such combination is called *symbiosis*.

Certain anaerobic bacteria grow in the presence of oxygen if other particular varieties of aerobic bacteria are present.

Attenuated tetanus bacilli become virulent if cultivated with *bacterium vulgæ*. Again, complicated chemical changes, as the decomposition of nitrites with the evolution of nitrogen cannot be accomplished by certain bacteria severally, but jointly, this is quickly brought about.

Pfeiffer has shown that certain chemical substances (foods, albumins, etc.), attract bacteria (*positive chemotaxis*), while other substances, as turpentine, repel them (*negative chemotaxis*).

Oxygen repels anaerobes and is particularly attractive to aerobes.

AGENTS PREJUDICIAL TO THE LIFE OF BACTERIA.

High temperatures are surely germicidal: 60°C. coagulates mycoprotein of bacteria and other common albumins. The degree of temperature at which bacteria are killed is called the *thermal death-point*. Most vegetative forms die after a short exposure at 60°C. , though some require a higher temperature, e.g., tubercle bacillus.

Spores resist boiling, often for hours. Spore-bearing bacilli from

the soil often survive a temperature of 115° C. moist heat (steam), from thirty to sixty minutes. Bacteria resist dry heat of 175° C. from five to ten minutes.

Cold inhibits bacteria; destroys some; but is not a safe germicidal agent, as typhoid bacilli have been isolated from melted ice in which they had been frozen for months.

Ravenel exposed bacteria to the extreme cold of liquid air (—312° F.) and found that typhoid bacilli survived an exposure of sixty minutes; diphtheria, thirty minutes, and anthrax spores, three hours; during this exposure, however, many were destroyed. In each instance, vegetative forms grew after the exposure.

Light is inimical to the life of bacteria; direct sunlight being the most germicidal, as it destroys some, reduces the virulence of others, or interferes with the chromogenic properties. Typhoid, cholera, diphtheria, and many other organisms are killed after an hour or two's exposure to bright sunlight. The ultraviolet or actinic rays are the efficient ones. If free oxygen is excluded, the germicidal action is very materially reduced. Sunlight acting on culture media (free oxygen and water being present) produces after ten minutes, peroxide of hydrogen. This action of light on bacteria has been extensively used, notably by Hansen, as a therapeutic measure for the cure of bacterial skin diseases, especially lupus. Diffuse sunlight, electric light, Roentgen-rays, continuous and alternating currents of electricity, are also more or less germicidal. Anti-septics, such as metallic salts, formalin, carbolic acid, cresol, mineral acids, and essential oils, are powerful germicides; some even in high dilution.

According to Koch, absolute alcohol, glycerine, distilled water, and concentrated sodium chloride solution do not affect anthrax spores, even after acting on them for months. Halogen elements (iodine; bromine, chlorine) are the most powerful germicides.

Free acids and alkalies must be very strong to act as disinfectants. Excessive amounts of sugar, salt, glycerine, and the pyroligneous acids act as destroyers, or inhibitors to bacterial growth in food stuffs.

Metals act as lethal agents in the presence of light and water, by

forming metallic peroxides, which either destroy the vitality of bacteria or hinder their growth. Silver, zinc, cadmium, bismuth, and copper, have this action. Consequently silver wire, or foil, are used in surgery because of their anti-septic action. Metallic fillings in teeth prevent the growth of bacteria that cause caries.

Certain *cells* in the *bodies* of animals (leucocytes) and some elements of the blood serum, being bactericidal, are a powerful means of internal defense against infection.

If the water of the cytoplasm of bacteria cells is dried out, the vitality of the organism suffers. The length of time required for drying varies, anthrax spores resisting the process for over ten years. Ancient methods of preserving foods from putrefying, and which are still in vogue, depend upon the employment of some of these agents, which are prejudicial to bacterial life. Meats are salted, pickled, dried, or smoked. Fruits are dried, pickled, or immersed in strong saccharine solution, in order to preserve them from decay, in every instance, the absence of moisture, the excess of salt, sugar, or vinegar, or the pyroligneous acid from the smoking, prevents bacterial growth, and consequently, decay of the food stuff. The *products* of bacterial growths often inhibit, or destroy, the cells that made them, as well as other bacteria. *B. Pyocyaneus* and *S. Cholerae*, have this property of secreting autolytic ferments.

CHAPTER II.

PRODUCTS OF BACTERIAL ENERGY.

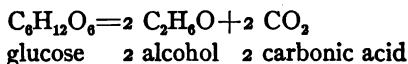
According to their chemical activities, bacteria are arbitrarily divided into the following classes:—

*Photogens**Chromogens**Zymogens**Saprogens**Aerogens**Pathogens*

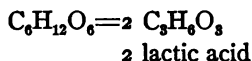
Photogens are those bacteria of the sea, putrefying flesh, and damp rotten wood, that produce a faint phosphorescence.

Chromogens are bacteria that produce colors as they grow, notably among which may be mentioned the *Staphylococcus Aureus*, that are golden in hue; *B. Pyocyaneus*, of a greenish-blue; and *B. Prodigiosus* which appears a brilliant red.

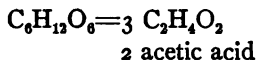
Zymogens are the bacteria of fermentation, which is the chemical transformation of carbohydrates by the action of bacteria, with the evolution of CO_2 , CO & H . Such bacteria are useful in the arts for the production of alcoholic beverages, wine, beer, etc. Through the actions of these organisms grape sugar is converted into alcohol, lactic acid, and acetic acid.



or



or



From the bodies of ground yeast cells a soluble ferment, *Zymase*,

has been expressed, which causes alcoholic fermentation of cane, and grape sugars. This fact proves that fermentation is not necessarily a vital process.

Enzyme Production by Bacteria.—Ferments of great variety and power are formed by the zymogens, as *proteolytic*, which dissolve proteids, such as casein; *tryptics*, gelatine liquefiers; *diastatics*, which convert starch into sugar; *invertin*, which changes cane sugar into grape sugar; ferments that curdle the casein of milk; as well as *bacteriolysins*, which destroy bacteria; *hæmolysins*, the dissolvers of the red blood cells (streptolysin); and many poisons that have a most deleterious effect upon body tissues. Hæmolysins are produced by *B. Pyocyaneus*, *B. Typhosus*, *B. Tetanus*, *B. Megatherium* and *Streptococcus pyogenes*.

Organized ferments (bacteria, yeasts) differ from the *unorganized* (pepsin, diastase). The latter "exercises solely a hydrolytic action" (Fischer), causing the molecules of insoluble compounds to take up water and to separate into less complex molecules of a different constitution, which are soluble in water. The organized ones act differently. Highly complex molecules are split up, and numerous substances of a totally different character are formed with the evolution of gases and by-products. (Fischer.) The reason for this is, perhaps, to be found in the supposition that the bacteria abstract oxygen for their own use, and thus cause the atoms to unite into an entirely different substance. According to the above named investigator, it is not possible to express such chemical changes by a simple equation. Experiments have shown that *B. typhoid* and *pyocyaneus* are able to split up olive oil or fat, and produce glycerine and fatty acids, thus making them accessible to fermentation (Fischer).

Saprogens produce putrefaction, which is the chemical transformation of albuminous bodies with the evolution of nitrogen, and of alkaloidal substances, known as **ptomaines**. Aromatic elements are also produced, such as indol, phenol, kresol, odors, etc. Complex bodies known as toxins, or toxalbumins, plasmins or bacterial proteids, are evolved by the pathogenic bacteria. The notable

toxins are those derived from the diphtheria and tetanus bacilli which are extremely sensitive to the action of heat, light, reagents, and oxygen, and therefore, are not thermostabile. Most of the toxins are soluble in water.

Among the plasmins we have those poisonous substances contained within the pathogenic bacteria themselves; notably Koch's "T R," a new tuberculin; the mycoprotein from cholera spirilla, and typhoid bacilli.

The *Bacterial proteins* are *thermostabile*, and produce intense local or general reaction if injected under the skin. They are often valuable agents in diagnosis and therapy. Koch's *old tuberculin* and *mallien* belong to this group. Ehrlich finds that the diphtheria toxin is a highly complex substance consisting of toxins, toxons, toxoids, epitoxoides, protoxoides, syntoxoides, and proto-toxoids, etc. According to Brieger and Cohn tetanus toxin does not dialyse. Watery solutions are not affected by heat, but in time lose their poisonous properties. The addition of H_2S . or alkali or acids injures it. Dry toxin withstands a temperature of $70^{\circ}C$. for a long time. It is, however, slowly converted into an inert substance, even if light, air, and moisture are excluded. Bile and pancreatic juice destroy the efficiency of toxins. If tetanus toxin is ingested it escapes, unabsorbed, through the anus. The extreme poisonous quality of tetanus toxin is marvelous, .00005 mg. will kill a mouse, a man weighing 150 lbs. at this rate would be killed by .23 mg. Whereas, from 30 to 100 mg. of strychnia are necessary to do this.

Diphtheria toxin may be precipitated in bouillon by absolute alcohol. Ammonium sulphate added to tetanus bouillon causes the toxin to rise and float on the surface of the fluid. Tetanus toxin is best preserved in a dry dark place, and in a desiccated condition. It is a complex toxin, consisting of at least two components: tetanospasmin, and tetanolysin, each of which has a specific effect upon different tissues of the body.

Bacteria of fermentation, and others, produce various gases:— CO_2 CO . H . N . NH_3 ., marsh gas and HS_2 . Many forms of bacteria

are differentiated from each other by the kinds of gas that they generate; the proportionate amounts of different gases; and from the fact that one form is aerogenic, and the other not. Again certain bacteria ferment but one or two sugars, while another strain or kind will ferment other different sugars.

Pathogens.—If the tissues are receptive to bacteria, and if the latter, in any way, injure the tissues, then the invading organism is called *pathogenic*. Theoretically the tissues of the body are sterile. But as a matter of fact, isolated pathogenic bacteria such as colon and diphtheria bacilli, streptococci, and pneumococci, are often found in the tissues and cavities of the body, and yet they cause no pathogenic changes or symptoms. The blood during life is sterile.

Colon bacilli have been found in the liver and kidneys of non-diseased cadavers, shortly after death, encapsulated, which showed that they had been there some time. Sixteen hours after death the blood and tissues teem with bacteria that have wandered in from the intestines.

Bacteria may cause disease in several ways, mechanically: a clump of bacteria may plug a capillary; or simply overwhelm the tissues and absorb the oxygen (anthrax); they may cause new growths (tubercle); or false membranes to form in the larynx causing suffocation (diphtheria); ulceration of heart valves causing cardiac insufficiency; thrombosis in the veins and arteries; pus formation; or, by generating toxins that cause anæmias, or degeneration of important elements of the nervous system.

The tissues of certain animals are receptive for particular bacteria, and the latter are therefore pathogenic to that animal. *B. of swine plague* is pathogenic to swine, but not to man. *B. typhoid* is pathogenic for man, but not to swine.

Reduction of nitrates. Some bacteria of the soil have the property of reducing nitrogen compounds, especially nitrites and ammonia, in which latter form they are easily assimilated by plants. Soils can be enriched by sowing them with nitrifying bacteria. The

United States Department of Agriculture supplies such cultures to agriculturalists. Bacteria not only reduce nitrogen compounds, but may assimilate nitrogen from the air and apply it to the nourishment of plants.

CHAPTER III.

INFECTION.

Infection means the successful invasion of the tissues of the body by either animal (protozoa, vermes) or vegetable (bacteria, and moulds) organisms. To successfully infect the body, bacteria must enter the tissues, be of sufficient number, find the tissues receptive, and continue to multiply.

The skin, mucous membranes, and the various cavities of the body connected with the outside air, teem with countless bacteria at all times, many of which are pathogenic, yet there is no infection, because the tissues are not invaded. Again, there can be no doubt that highly pathogenic bacteria enter the tissues of healthy people at times, in small numbers, and yet no disease is produced, because of their scarcity, or by reason of the tissues not being receptive. Infection implies not only invasion of the body, but injury to the tissue. Certain bacteria may invade a body, and yet create no harm. These bacteria may enter dead or dying body tissues, and secrete poisonous substances (toxins) which may be absorbed, and produce pathologic symptoms known as *Sapremia*. Clots of blood in the parturient uterus, and gangrenous limbs may be invaded by strict saprophytes incapable of life in living tissues, and yet cause much harm by the absorption of their products.

Depending upon their ability to grow in the body, bacteria may be divided into (1) purely saprophitic; (2) occasionally parasitic; and (3) purely parasitic.

Purely saprophitic cannot live in tissues at all; those that are occasionally parasitic lead a saprophitic existence in the soil or water, and yet may invade the body, and produce disease:—the tetanus and

malignant cedema bacilli are examples of this group. Those bacteria that are purely parasitic are only known as they exist in the tissues of the infected host, and have no outside existence at all. The bacilli of leprosy, and the spirillum of relapsing fever belong to this group.

Kruse's Scheme Illustrating the Action of Various Parasitic Bacteria.

Parasitic Bacteria.

A

Occasionally parasitic.
Such as the Tetanus bacillus.

1. *Local infection due to the ability of the organism to take on unrestricted growth.*
 - a. *Surface inflammation, boils; staphylococci.*
 - b. *Surface inflammation with extension of continuity; erysipelas; streptococci.*
 - c. *Surface infection with marked toxin products; diphtheria.*
 - d. *Deep focal inflammation; tubercles.*

B

Always parasitic only found in the lesions of disease. Such as the B. Tuberculosis.

2. *General infection of unrestricted growth.*
 - a. *By continuous infection; glands.*
 - b. *Metastases, as in pyæmia.*
 - c. *By universal rapid growth and invasion, as in sepsis and anthrax.*

Koch's Postulates.

In order to prove that a certain organism is the infectious agent of a given disease, Koch has devised four postulates which the given organism must fulfil before it can be considered the cause of the disease.

1. The organism must be found microscopically in the tissues of the animal having the disease, and its position in the lesion should explain the latter.

2. It must be isolated in pure state from bodies of the diseased animals.

3. And then it must be grown for successive generations in culture media.

4. If injected into a healthy animal, or animals, it must produce the same disease, and be found in the lesions of the disease in the animal's tissues.

Some of the many organisms that certainly fulfil these conditions, are as follows:—

<i>Streptococcus Pyogenes (Sepsis).</i>	<i>Actinomyces</i>
<i>B. Tuberculosis.</i>	<i>B. Diphtheria.</i>
<i>B. Anthrax.</i>	<i>B. Tetanus.</i>
<i>B. Mallei.</i>	<i>B. Malignant Oedema.</i>
<i>B. Bubonic Plague.</i>	<i>B. Malta Fever.</i>
<i>B. Typhoid.</i>	<i>B. Dysentery.</i>
<i>Spirillum Cholera.</i>	<i>Meningococcus.</i>
<i>Pneumococcus (Pneumonia).</i>	<i>Gonococcus.</i>

There are several other organisms that are considered to be the cause of specific disease, but they do not fulfil the postulates. Among these are:—

- B. Lepra.*
- Spirillum of Relapsing Fever.*
- The Organism of Scarlet Fever (Protozoans).*
- The Protozoa of Malarial Fever.*
- Amæba Coli.*
- Treponema Pallida of Syphilis.*

In rheumatic fever, measles, whooping-cough, mumps, yellow fever, typhus fever, chicken-pox, rabies, and dengue, the specific cause has, thus far, eluded discovery. In the case of yellow fever (Reed and Carroll) and hog cholera, it has been found that the cause of these diseases resides in the blood, and if the serum of the latter is carefully filtered through a Berkefeld filter, it is still capable of producing the disease in susceptible animals. Careful microscopic search fails to show any bodies in the serum that might be considered the agents of infection, and it is thought that these organisms are submicroscopic.

If the invading organism is a pure saprophyte the various forces for internal defence immediately act upon and destroy it. If it is pathogenic for other animals, their defensive agencies have no effect upon it in their tissues, but in the human body the bacteriolysins dissolve it, or the phagocytes devour it and carry it away. The liver, according to Adam, destroys bacteria absorbed from the intestines at once.

Bacteria are disposed of in divers ways, by means of the lymph channels. They are carried to the various mucous surfaces of the body, intestinal and bronchial. During typhoid fever, the typhoid bacillus is often found in the urine. The kidneys at least allow the escape of some organisms of the blood. Pathogenic bacteria are often discharged from the body in feces, pus, sputum, and in scales in the desquamating skin diseases.

To successfully inoculate a guinea pig with tuberculosis, the tubercle bacilli should be injected beneath the skin.

In working with infections produced by the *B. proteus vulgaris*, it was found by Watson—Cheyne that 6,000,000 bacilli injected under the skin, did not produce any lesion; 8,000,000 formed an abscess; 56,000,000 gave rise to a phlegmon; and 225,000,000 were necessary to cause death in two hours.

In experimenting with the *staphylococcus aureus*, it was found that 250,000,000 were required to cause an abscess; and 1,000,000,000 were needed to cause death. The internal powers of defense

were able in each case to cope with or limit the action of a few million to a certain locality, but could not withstand the injection of overwhelming numbers, which caused the animal's death.

Bacteria to be successfully infectious must be virulent. Their virulence can be lessened by cultivation at a higher temperature than the body, 42.5°C. to 47°C. ; by drying; the exposure to light; the action of chemicals; compressed oxygen; and by passing the organism through the bodies of non-susceptible animals. The attenuation or weakening of the pathogenic powers of bacteria is useful for the production of various vaccines which are valuable in preventative medicine.

By growing the anthrax bacillus at a high temperature, 42.5°C. , it becomes so avirulent that it is incapable of destroying sheep or rabbits. It is then used as a vaccine to prevent infection with virulent bacilli. By exposing the spinal cords of animals dead from hydrophobia to the action of drying for various periods, Pasteur was able to attenuate the virus, so that it would not produce hydrophobia, but on the contrary, it, by repeated inoculation, caused immunity. The inoculation of monkeys (which are non-susceptible) with hydrophobia virus attenuates it. The growth of the small-pox organism in the cow, causing cow-pox, so reduces the virulence of the germ that it is incapable of producing small-pox in man, but only vaccinia; infection with this gives immunity against small-pox. The flesh of animals that have died from *quarter-evil* is so changed by heat and desiccation that if it is injected into susceptible animals, they do not succumb but are vaccinated against infection with the virulent organism.

The malignancy of bacteria may be heightened in various ways: (1) By passing them repeatedly through the bodies of susceptible animals; (2) by cultivation in culture media in collodion sacs placed in the abdominal cavities of animals; (3) by injections mixed with other injurious substances, such as lactic acid, and the metabolic products of foreign bacteria. Cultures of pneumococci may be made so virulent by the first means that only one pneumococcus is

capable of setting up a fatal septicæmia in a rabbit. By injecting attenuated diphtheria bacilli with streptococci into a rabbit, the virulence of the bacilli can be raised, as mixed infection often adds to the virulence of an organism. Malignant streptococcic infection added to virulent diphtheria infection, greatly increases the severity of the disease.

The secondary streptococcic infection in small-pox and in phthisis complicates the primary infection and frequently causes death of the individual affected. The hectic fever and sweats of phthisis are due to this secondary infection. Combinations of diphtheria bacilli and pneumococci increases the virulence of the latter. The transference of infection agents from one person to another during an epidemic increases the virulent action of the organism by reason of the rapid passage from individual to individual. Two infections may occur simultaneously, each preserving separate characteristics, and perhaps aggravating each other.

The avenue of infection and the tissues infected alter the type of the disease exceedingly. Streptococci invading the tonsils cause tonsillitis, but the same organisms entering the skin cause erysipelas or phlegmons; or if the uterus is infected after the birth of a child the disease is still different and more serious. If the tubercle bacilli enters the skin it produces lupus; if swallowed it causes ulceration of the bowels, and subsequently invades the peritoneum; if inhaled, tuberculosis of the air passages, phthisis, or tubercular laryngitis may follow. If cholera spirilla be injected into a vein of a guinea pig, it may develop choleraic septicæmia; if they are injected into the peritoneal cavity, a choleraic inflammation of the peritoneum is produced, and not a septicæmia. Pneumococci if injected into a vein cause a rapid septicæmia, or they may give rise to abscesses anywhere in the body. Like streptococci, they may be the cause of inflammation in any tissue, particularly serous membranes, and show different clinical entities, according to the organs involved, and the morbid anatomy and physiology produced. The fatality of a bacterial infection varies with the avenue of inoculation: it is safer to

have a skin infection than a meningeal, or endocardial one, not only from the likelihood of rapid toxin absorption, but from purely mechanical damage, as pressure and interference with vital functions by inflammatory products such as exudates, tubercles, serum and pus. The injection of pneumococci under the skin of a dog had a more rapidly fatal effect than when they were injected into a vein, according to Klemperer.

Local immunity to infection. There is evidently more resistance offered by the liver against invasion than by the peritoneum. It is not likely that a man would contract typhoid through skin infection, nor is it probable that he would contract tetanus by swallowing tetanus bacilli, but the reverse of these conditions certainly produces infection.

Infection may be caused from without the body, or from within. Lockjaw, sepsis, hydrophobia, or anthrax may follow injuries from rusty nails, splinters, weapons, unsterile fingers, or instruments. Personal intercourse, bites, kisses, sexual intercourse, association with persons suffering from exanthematous or contagious diseases.

The bites of insects that are intermediate hosts of infectious agents (plague bacilli, malarial organisms, etc.) are sources of infection from without, as is also the ingestion of infected food or water.

Infection from within may be caused by the migration of bacteria from the skin inwards, or from any of the mucous membranes, on which, and in which many pathogenic bacteria at all times may be found.

Bacteria from the mouth, stomach, intestines and the rectum may invade the tissues and the blood under certain conditions. This is particularly the case during the last stages of diseases, not necessarily infectious, such as chronic heart disease, kidney disease, or diabetes. Vital resistance is much lowered, and intestinal bacteria, invading the tissues in enormous numbers, set up what is known as *terminal infection*, which is often the immediate cause of death.

The stomach with its gastric juice, containing during digestion .2 percent to .3 percent of hydrochloric acid, guards the lower ali-

mentary tract against infection. A great many bacteria are ingested with foods, particularly with milk, cheese, and over-ripe fruit. These in the most part are quickly destroyed by the hydrochloric acid. When the stomach is diseased and the contents become stagnant, as in stenosis of the pylorus, and in carcinoma, when HCL is diminished, or absent, fermentative bacteria give rise to great amount of gas, and lactic acid, to the great discomfort of the patient. The normal acidity of the stomach is a great safe-guard against infection with cholera. If tubercle bacilli are swallowed, and if infection occurs, the lesion is not always localized to the alimentary tract. Lesions of the lymph glands, peritoneum, bones, and nervous tissues often follow the ingestion of these organisms. Dogs fed on soup containing great numbers of tubercle bacilli, and then killed three hours after, were found to have bacilli in the thoracic duct. Chyle from the duct, injected into guinea pigs, caused tuberculosis in them (Nicolas and Descos). Cholera and typhoid organisms thrive in intestinal contents, elaborating poisons which greatly depress the individual.

The interior of the uterus, the bladder, urine, and deep urethra, are generally sterile in health.

The placenta is an avenue of infection in several diseases: notably small-pox, anthrax, glanders, typhoid fever, and sometimes tuberculosis pass through the placenta from mother to foetus. Streptococci may pass through the placenta of a woman with ante-delivery sepsis and cause peritonitis in the child. Recurrent fever has been transmitted from mother to foetus, and the specific spirillum has been detected in the latter's blood.

A case has been recorded in which a woman suffering from pneumonia gave birth to a child, which died thirty-six hours afterwards, and autopsy revealed a consolidation of the lower left lung, and microscopic examination discovered pneumococci. A hydrophobic cow was delivered of a calf that developed rabies three days after birth.

McFarland divides microbic infection in three heads:

Phlogistic. Characterized by restricted growth and local irritation.

Toxic. Characterized by restricted growth and toxic dissemination.

Septic. Characterized by unrestricted growth in the blood and lymph. In the three groups, the damage is done ultimately, by metabolic products acting on the tissues. If the product is not soluble the harm done is purely local, as in the formation of tubercles by the toxin of the tubercle bacilli.

If the growth is restricted, as in tetanus and diphtheria, the toxin being soluble and diffusible, harm is done to tissues remote from the infected area.

Anthrax and streptococci and other pus organisms by rapid increase in the blood eventually infect all the tissues.

Combinations of these forms of infection may be at first confined to some particular area like the pneumococcus, which are generally restricted to the lungs at the outset, but ultimately they infect the blood, causing septicaemia and localized lesions in more or less remote parts, such as the veins of the leg, or inflammation of the meninges.

Soluble products of bacterial activity which are alkaloidal (basic), crystalline in character, and mostly poisonous, are known as *ptomaines*, or putrefaction alkaloids. They are highly complex in chemical structure, and are difficult to isolate.

Certain albuminoid bodies, products of bacterial activity, known as toxins, are produced by several pathogenic bacteria.

Those that are essentially bound up in the protoplasm of the bacteria itself, are known as intracellular toxins, or bacteria plasmins. The tubercle bacillus, and other members of the acid-fast group, the colon and typhoid bacillus, and the cholera spirillum all contain these. They may be extracted by freezing the organism with liquid air, and grinding it while frozen and brittle, or by simply grinding it with sterile sand and water. The new T.R. tuberculin belongs to this group.

Bacterioprotein are albuminous bodies produced by bacteria that are not altered by heat, and which produce fever and inflammation. The best examples of these are mallein, a product obtained from old cultures of glanders bacilli, and the original, or old tuberculin of Koch.

Toxins or **toxalbumins** are soluble bacterial products which are removable by filtration from the bacteria, and which are thermolabile. The tetanus and diphtheria toxins belong to this class.

These various poisons produce many of the clinical pathological entities and symptoms, known to physicians. Their highly complex molecular structure enables a group of atoms in the toxic molecule to unite with a certain other group of atoms in the protoplasmic molecule of a body cell. The latter is either killed outright, or else is stimulated to produce other free groups of combining atoms (lateral chains) which may unite with other toxic groups.

Various kinds of cells are attacked in infective processes. Leucocytes may be degenerated, forming pus; red blood cells may be dissolved, causing anæmia; important nerves may be degenerated; or muscle fibers of the heart may undergo fatty degeneration and die. Again, mechanically important serous cavities may be filled with serum, interfering with normal functions of the enveloped organs. The heart orifices may be closed partially or emboli may form, or false membranes block the air passages, and a hundred other pathological changes may be wrought by these toxins.

If toxins are injected into the body with the specific organism producing them, the effect of the latter is greatly increased. Tetanus spores, washed free of toxins, if injected, are incapable of setting up tetanus.

Most toxins are easily decomposed by sunlight, air, and heat. Absolute alcohol separates the active principle from the bouillon in which it grows. Ammonium sulphate also separates the toxins of tetanus and diphtheria bacilli, which float on top of the fluid, from which they may be collected, dried and powdered, and in this state may be kept much longer without deteriorating into inert substances.

Small quantities of bile and pancreatic juice destroy the toxic properties of diphtheria and tetanus toxin.

If toxin and anti-toxin (see immunity chapter) are mixed in relative properties, chemical neutralization takes place. Since the toxins cannot be isolated in a chemically pure form, their exact composition cannot be known, except by studying their effects upon animals and animal tissues. Hence, when anti-toxin, added to toxin in a test-tube is injected into an animal, and no harm results, it is rightly assumed that the toxin is neutralized, and both are chemically bound; yet if fresh toxin is added to the mixture, it is no longer neutral.

If the toxin of the pyocyaneus and the anti-toxin be mixed so that they neutralize each other, and if the mixture is heated, the neutralization disappears, and the mixture becomes toxic again. That the union is a chemical one, may be inferred from the fact that it is more rapid in concentrated solution than in weak, and is much quicker when warmed than when cold, and it follows the law of multiples, one part toxin neutralizing one part of anti-toxin, and ten parts of toxin neutralizing ten parts of anti-toxin. All this is in accord with chemical laws. Toxins sometimes degenerate into what Ehrlich has called *toxoids*, substances that bind (unite with) anti-toxin just as effectively as toxins, while they are not poisonous, yet may stimulate healthy cells to secrete anti-toxins if they are injected into the body of experiment animals.

More is known about the toxins of diphtheria and tetanus bacilli than of any other. Diphtheria toxin has numerous component substances, one of which is the toxin that causes the acute phenomena of diphtheria intoxication. Another, *toxon*, causes cachexia and paralysis some time after infection.

Tetanus toxin is composed of two substances; tetanospasmin and tetanolysin. The first unites chemically with the motor elements of the nervous system, producing degeneration and causing tremendous contractions of the muscles governed by the nerves involved. The second has the property of dissolving tissues, such as blood cells.

Tetanus toxin travels from the infected site to the cord by way of the nerves; it is exceedingly poisonous, a single prick of the finger with a needle moistened with toxin, has induced tetanic symptoms.

If tetanus toxin of known strength is mixed in a test-tube with fresh brain substance of a guinea pig, the toxin is no longer toxic for guinea pigs. This shows that there is a chemical union of the toxin and the cells of the brain. Cells of other organs had no such effect. This explains specific action of tetanus upon nervous tissue.

Aggressins.

If tubercle bacilli are injected into the abdominal cavity of a guinea pig, rapidly fatal tuberculosis is produced. If the exudate produced in the peritoneum, consisting of lymphocytes, is sterilized and injected into another guinea pig, together with some virulent tubercle bacilli, the animal will succumb in twenty-four hours. If the exudate alone is injected no effect will follow, if bacilli alone are injected, a tuberculous peritonitis will be produced in a few weeks. It is the exudate plus bacilli that does the harm. The exudate is, in this instance, the aggressin. Bail, who originated the doctrine of aggressins, believes that a bacteriolysin is produced, which, acting on the bacilli, liberates an endotoxin, which paralyzes the polynuclear leucocytes, inhibiting their action as phagocytes.

By heating the exudate to 60° C. the aggressins are increased in activity, and it has been found that small amounts are relatively stronger than larger ones.

This phenomenon has been explained by Bail in this way. He assumes that there are two substances in the exudate, one is thermolabile, which prevents rapid death, the other is thermostabile and this is favorable to rapid death.

Bail assumes that a tubercular cavity in an animal contains a great amount of the aggressin, which prevents chemotaxis of the polynuclear leucocytes, but not of the mononuclear or lymphocytes.

In the peritoneal cavity without aggressins, into which tubercle bacilli have been injected, an active phagocytosis at once is begun by the polynuclears, and the injected bacilli are in a great measure destroyed, and those left develop more slowly, producing a tuberculosis in normal course of time.

This aggressin theory has been applied to other infections with like results, notably in pneumococcus, typhoid, dysentery, and plague infection.

CHAPTER IV.

IMMUNITY.

By immunity, is understood the inherent power of a living body to successfully withstand the invasion of infective agents, e.g., bacteria, or such deleterious and toxic substances as toxins, drugs, complex poisonous albumins, snake venom, foreign blood sera, etc.

The following tables will, perhaps, be helpful in the study of the subject.

1. Immunity	$\left\{ \begin{array}{l} \text{Natural} \\ \text{Acquired} \end{array} \right.$	$\left\{ \begin{array}{l} \text{Racial immunity} \\ \text{Inherited immunity} \\ \text{Active immunity} \\ \text{Passive immunity} \end{array} \right.$
2. Immunity	$\left\{ \begin{array}{l} \text{Anti-toxic} \\ \text{Anti-bacterial} \end{array} \right.$	

It is a well known fact that one attack of an infectious disease generally protects an individual against a subsequent attack. It has also been known for centuries that the human system, by first taking very small doses, and gradually increasing them, can be so accustomed to poison, that large, and otherwise deadly quantities may be taken at one time with impunity. Among the poisonous substances to which men can accustom themselves are:—tobacco, morphia, arsenic, and alcohol. Animals treated in a like manner also become immunized to powerful toxins, snake venom, etc.

Natural Immunity.—The hog is immune to snake venom; the chicken to tetanus. Man is immune to hog, or chicken cholera. The negro is not so susceptible to yellow fever as is the white. Ani-

mals cannot be infected with scarlet fever, malaria, and measles. Young adults are more susceptible to typhoid fever than are elderly ones. Infants are exceedingly prone to suffer from milk infection while older children are not. Certain diseases are known as children's diseases, because adults rarely have them. Again, one individual may contract a disease, while another exposed at the same time will not.

Acquired Immunity.—Actively acquired by infection. One attack of yellow fever immunizes the individual against subsequent attacks. Vaccination actively immunizes against small-pox.

Passively acquired. Actually injecting protective substances (anti-toxic sera) into the blood. The immunity against a given disease (diphtheria) resides in the anti-toxic sera.

Immunity is nearly always relative. A small quantity of toxin may be innocuous, while a large quantity may cause a fatal toxæmia.

There have been several theories advanced to account for the various phenomena of immunity.

Exhaustion Theory.—Pasteur conceived that bacteria as they grow in the body, used up or exhausted something vitally necessary to the subsequent growth of that particular kind of bacteria.

Retention Theory.—It was held that some noxious agent was retained by the body which prevented the further growth of bacteria.

The modern conception of immunity deals with two theories. The theory of phagocytosis of Metchnikoff, which may be termed the cellular or biologic one. And the lateral-chain, or the humoral or chemical theory of Ehrlich. Both of these are extremely ingenious and explain satisfactorily why certain bacteria are unable to infect the body, and why, the body once infected, cannot, in many diseases, be again infected. Furthermore these theories make it clear to us why the body tissues during life do not fall an easy prey to many putrefactive bacteria, as after death.

Phagocytosis is essentially a theory of cell devouring. Leucocytes which are white mobile cells of the blood, and other fixed cells, de-

fend the body against infection by devouring the invading agents of disease. (Fig. 12.)

Metchnikoff considers the subject of phagocytosis under three aspects: 1 Nutritional. 2. Resorptive. 3. Protective.

Nutritional.—Amœba and certain other unicellular vegetable organisms belonging to the *myxomycetes* possessing amœba properties and having the faculty of throwing out pseudopodia or protoplasmic arms, acquire their food by enveloping smaller organisms,



FIG. 12.—Phagocytosis. Gonococci in leucocytes in pus from gonorrhœa. (Kolle and Wassermann.)

and other nutritious matter which they absorb. Certain intracellular ferments, which they possess, digest fibrin and gelatine, and convert starch into sugar. These cells protect themselves against inimical micro-organisms by enveloping and digesting them.

They are attracted by food and moisture (called *positive chemotaxis*) and repelled by strong solution of salt, poisons, etc. (*negative chemotaxis*).

Higher in the animal scale among the multicellular organisms, the cells of the intestines have the property of absorbing and digesting food. These fixed cells are called *sessile phagocytes*. Still higher

in the scale (man) certain digesting cells are present in the digestive tract, which are incapable of absorbing food. They, however, secrete ferments which digest gelatine and fibrin, and convert starch into sugar. They are not directly concerned in the nutrition of the organism. Cells of a protecting character in man are either microphages, or macrophages. The microphages are the polynuclear leucocytes, which are concerned in the protection of the organism against acute infections, the bacteria of which they take up and devour. The macrophages consist of the large lymphocytes, the endothelial cells, and some connective tissue cells, which take up foreign bodies. Both of these classes contain ferments; microcytase being found in the microphages; and macrocytase in the macrophages. The latter absorb connective tissue cells through their particular ferments, and are active in immunizing against tuberculosis. These cells perform various functions in the body. When the tissues are invaded with bacteria, the blood shows an increase in the number of these microphages, which have been called the "hygienic police." Summoned to repel invasion, they leave the lymph stream for that of the blood. All the phenomena of leucocytic emigration in inflammation is a manifestation of positive chemotaxis. During practically all the infections, the peripheral blood contains an excess of leucocytes over the normal amount per cubic millimeter (7600). In exceptional infections, typhoid fever, influenza, measles, and tuberculosis, there is no such increase, or leucocytosis. In malaria (not a bacterial infection) there is also no leucocytosis.

Metchnikoff has described a process in which the phagocytes undergo what he calls phagolysis. The ferment, *cytase*, is discharged and acts extracellularly, as in the hæmolysis of foreign red blood cells in the peritoneum of a guinea pig. This phagolysis in dissolving of the leucocyte is the cause of the chemical phenomena (so he avers) which cannot be ascribed merely to phagocytosis. Metchnikoff further claims that both phagocytosis and phagolysis, either severally, or in combination, are responsible for natural or acquired immunity.

In the case of acquired immunity, it is supposed that the leucocytes

become educated. Regarding the toxins against which animals can be immunized by gradually increased doses, it is held by him that the educated leucocytes neutralize the poison by their secretions.

In the case of anthrax infection, animals infected with virulent cultures of this organism quickly succumb, without exhibiting any leucocytosis (negative chemotaxis).

If the animal has been previously immunized with attenuated culture the injection of a virulent culture is followed by an enormous outpouring of leucocytes at the site (positive chemotaxis), while if the site of the inoculation in the non-immune animal is examined, only a few leucocytes will be found, and some clear serum.

Toxins, if injected, cause a negative chemotaxis. If tetanus spores are injected into an animal, together with some toxin, the animal rapidly succumbs to tetanus, without evincing any leucocytosis. If the spores are washed free from toxin, and injected, active leucocytosis occurs and the animal survives.

A mixed infection of a highly virulent culture, and a non-virulent one, often hastens the action of the virulent one. It is supposed that the non-virulent bacteria engage the leucocytes, so that these cells cannot cope with the virulent ones.

Phagocytosis thus plays an important part in the protection rôle in natural immunity, but no satisfactory theory has yet been offered in explanation of the protective process in acquired immunity, at least against toxins and other soluble and unorganized poisons.

The **cellulo-humeral** theory claims the attention of most bacteriologists, as the probable explanation of the phenomena of immunity.

It is certain that cells, either sessile or mobile, and fluids, are important means of internal defense. In order that this theory may be comprehended, certain well known properties of normal and artificially immunized serum must be understood.

Alexins.—It has been found by numerous observers, that normal blood serum is germicidal for many bacteria, and the peculiarly active substance that is contained in the serum, was called by Buch-

ner *Alexin*. This dissolves bacteria and destroys them. It also destroys the red blood cells of other animals. The alexin of a dog's serum dissolves the red cells of a rabbit; it is therefore *hæmolytic*. It also is *thermolabile*, that is, its properties are destroyed by heat (55° C.). It is identical with the *complement* of Ehrlich, and the *cytase* of Metchnikoff.

The *complement*, as it will hereafter be called, takes, as already stated, an active part in bacteriolysis, or bacteria-dissolving, and in hæmolysis, or blood-dissolving, it is present in normal non-immune sera. R. Pfeiffer found that if some serum from a guinea pig immunized against cholera spirilla is injected into the peritoneal cavity of a healthy non-immune guinea pig, with some cholera spirilla, that the latter are agglutinated, and ultimately dissolved, having undergone bacteriolysis (Pfeiffer's reaction). The immune serum alone in a test-tube, with the cholera spirilla does not have this action, but if some normal guinea pig serum is added to the mixture, an immediate solution takes place, showing that the presence of both the normal serum containing the *complement*, and the immune serum, containing the immune body, or *amboceptor*, are necessary to complete the solution of the bacteria. If the *complement* is heated above 55° C. for an hour, solution does not take place, even if the immune serum is present, but, after heating the mixture, it may be *reactivated* by adding some fresh unheated *complement*.

The immune serum is not affected by heat, and is therefore called *thermostabile*.

These various reactions may be expressed concretely thus:

Bacteria + immune body = no solution.

Bacteria + complement = no solution.

Bacteria + immune body + complement = solution (Pfeiffer's reaction).

Bacteria + immune body + complement (heated) = no solution.

Bacteria + immune body (heated) + complement = solution.

The same phenomena in the blood of animals immunized against

the red blood corpuscles of another animal of foreign species have been observed.

If a rabbit is immunized with the blood of a dog by repeated and increasing doses, the serum of that rabbit will become *hæmolytic* to the corpuscles of the dog's blood if they are mixed, provided some normal rabbit's blood complement is added to the mixture.

Dog's erythrocytes + immune rabbit serum = no solution.

Dog's erythrocytes + immune rabbit serum + complement = solution.

Dog's erythrocytes + immune rabbit serum + complement, heated = no solution.

The immune body acts as a preparer of the corpuscles, or bacteria, so that the complement ~~can~~ act upon the cells. The reaction is very like the action of pepsin on fibrin. Hydrochloric acid must be present.

(1) Pepsin + fibrin = no solution or lysis.

(2) HCL + fibrin = no solution or lysis.

(3) Pepsin + HCL + fibrin = solution or lysis.

The HCL corresponds to the immune body.

In the case of hæmolysis, or bacteriolysis the action of the immune body is specific. The immune body of cholera spirilla will not prepare, or fix typhoid bacilli, so that they can be acted by the complement. Nor will the immune body of dog's erythrocytes prepare these of a pig, so that the complement may act on it.

A loose chemical union takes place between the bacteria and the immune body, but no such union occurs between the complement and the bacteria. The same chemical union occurs between the red cells and the immune body in hæmolysis, but not between the cells and the complement.

Ehrlich holds that there are many complements, each one different

from the other, and that their action is specific for the different kinds of bacteria or cells with which an animal may be immunized. Bordet and Buchner, on the other hand, maintain that there is but one complement.

The solution of any cells by immune bodies, or anti-bodies, as they have been called, is known as cytolysis. And cytolysins may be produced by making anti-bodies of nerve cells, leucocytes, epithelial cells, liver cells, as well as blood cells, by immunizing an animal against these different cells with repeated injections of the cells or emulsions of them.

Agglutinins are peculiar bodies which have the property of causing certain cells to agglutinate. One of the earliest manifestations of immunity of a certain serum to bacteria, or to blood cells, is this peculiar action of the serum causing either the bacteria or blood cells to clump together in masses. Part of Pfeiffer's reaction is the agglutination of the cholera spirilla in clumps before they are dissolved by the complement and immune body.

If the serum of a typhoid fever patient is mixed, even in high dilutions with some typhoid bacilli, the latter are clumped in isolated groups. Clinically this is known as the *Widal* reaction, and is the most reliable single sign of typhoid fever.

These agglutinins may be produced artificially by injecting large and increasing doses of bacteria into animals. After a time, in the serum of the rabbit, there develops a peculiar body which agglutinates typhoid bacilli, if they are brought in contact with it. Sera can be rendered so highly agglutinative as to produce this reaction even if diluted 100,000 times or more.

If an animal is immunized against spermatozoa, or the red blood cells of a foreign species, its serum becomes agglutinative to these cells.

Precipitins.—If a rabbit, or any other animal in fact, is immunized by repeated injections of blood foreign to it, peculiar bodies develop in its blood serum called precipitins, and these can be demonstrated by adding to the serum of the immunized animal in a test-tube a

minute portion of the blood against which the animal was immunized. As soon as the immunized serum and the specific blood are mixed, a precipitate at once forms. This is another phenomenon of immunity, and is of more than theoretical importance in medicine. The reaction is strictly specific; thus, if the serum of a goat is injected into a rabbit repeatedly the rabbit's blood will form a precipitate with normal goat's serum if the two are mixed in a test-tube. Old dried blood, semi-putrid blood, blood on whitewash, or rusty steel, even in minute quantities, if dissolved in salt solution, may be used to produce this reaction. In medico-legal matters, this test is of use for the identification of human blood. Naturalists also use this method for the differentiation of species. By many, the phenomenon of agglutination is supposed to be due to the formation of a precipitin, in the meshes of which bacteria or blood cells are caught and agglutinated, and that agglutination is but a modification of the formation of precipitins.

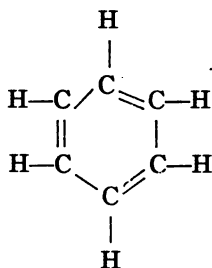
Anti-toxin formation is also another phenomenon of immunity. If an animal, such as a horse, receives numerous increasing doses of a given toxin, say that of tetanus, it, in a short time, becomes so accustomed to the poison, that it can withstand the administration of immense doses. (If these large doses had been given at first, they would have proved fatal.) If the horse is then bled, and its serum injected into rabbits or guinea pigs, they may receive shortly after, at one dose, enough toxin to kill ten such animals. The horse serum thus protected these animals against the toxin, as it was anti-dotal, or in other words anti-toxic. A chemical union occurs between the toxin and the anti-toxin, since, according to the law of multiples, a definite amount of anti-toxin unites with a definite amount of toxin. If ten times the amount of anti-toxin is used it will exactly neutralize ten times the amount of toxin, and the mixture becomes inert. Again, the union of the two substances follows well known chemical laws, whereby chemical union takes place more rapidly in concentrated than in dilute solutions, and when the solutions are warm. If the mixture of toxin and anti-toxin is heated, it, instead of being neutral,

becomes toxic again. This toxicity can be neutralized again by the addition of fresh unheated anti-toxic serum (*reactivation*).

The production of bacteriolysins, cytolysins, agglutinins, precipitins, and anti-toxins are manifestations of the activity of the immunized organisms. To further understand this activity, Ehrlich's *side-chain* theory of immunity must be comprehended. This is known as the chemical theory. To understand it fully some consideration must be given to the study of the toxin molecule. Ehrlich believes that each molecule of toxin is made up of two groups of atoms, constituting what is known in chemical nomenclature as lateral chains.

Many molecules are made up of a central body and lateral chain of atoms which are free to combine with other groups of atoms without disturbing the central body.

The benzol ring is very suitable for the demonstration of the relationship of the side-chain to the central body.

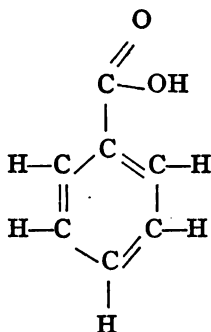


BENZOL.

The benzol molecule C_6H_6 is here represented graphically as a ring with a central nucleus of C_6 with lateral chains of H. connecting each atom of C.

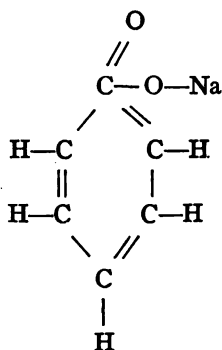
If one of these lateral chains H. is supplanted by the acid radical $COOH$. the benzol is converted into *benzoic acid* and its formula is represented thus:—

IMMUNITY.



BENZOIC ACID.

If to this acid radical of the benzoic ring, sodium hydroxid unites, supplanting an H in the OH of this radical, we have, instead of benzoic acid, *benzoate of soda*.



BENZOATE OF SODA.

It is thought that as the soda is brought in contact with the central nucleus of the benzol ring, so food stuffs unite with the central body of the cell molecule in the organism and nourish it.

In the case of toxin, the two lateral chains of its molecule are called *haptophores* and *toxophores*. The haptophores seize the lateral chains of the cell and the toxophores poison it.

Ehrlich conceived that cells were nourished by their lateral chains, each having a central nucleus with many lateral chains called *receptors* bristling all over it. Complex albumins, food stuffs or poisons (as the case may be) unite with it. This means a chemical union of a part of a cell with all or part of a group of atoms. But certain body cells are only capable of uniting with certain toxins. It is known that the toxin of tetanus has a chemical affinity for the nervous system and for its neural elements and not for liver or spleen cells.

The poisons of snake venom seem incapable of uniting with any cells of the pig; this animal is, therefore, immune to snake venom.

Now, as these toxins unite with the cells by means of the receptors, the cell is stimulated to produce an excessive number of these receptors, which are cast off and become free. Nature is very prodigal and whenever any of the tissues of the body have been injured, or there is a deficiency, an enormous excess of reparative cells is produced. Weigert first called attention to this phenomenon, which has been called Weigert's *over-production theory*. So when the haptophores of the toxin molecule combine with the receptors of the cell, the latter are incapable of any further union and are useless to the cell. Accordingly a great number of free receptors are generated, and floating in the blood, engage the haptophorous portion of the toxin. Thus the toxophore is neutralized and rendered innocuous. These free over-produced receptors constitute the anti-toxin. This is the essence of Ehrlich's theory. (Fig. 13.)

Through the process of time and oxygenation, the toxophorous group in the toxin becomes innocuous, and only the haptophorous group remains active; nevertheless the haptophorous group is able to combine with the receptors and to stimulate the cell into generating free receptors. This attenuated toxin is called by Ehrlich

toxoid. The receptors have been compared to a lightning rod, which if placed within a building would, if struck, cause disaster, while the same rod placed outside of the building, is a means of protection to the structure against lightning. This theory can be applied to the production of other anti-bodies. If blood cell, bacterial cell, or any animal fluid possessing a haptophore is capable of combining

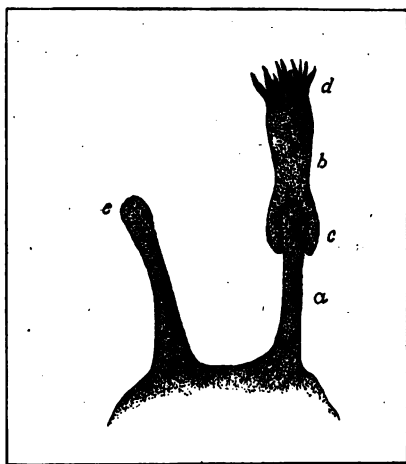


FIG. 13.—*a*, receptor on cell; *b*, toxin molecule; *c*, haptophorous portion of the molecule; *d*, toxophorous portion; *e*, receptor. (Williams.)

with side-chains (receptors) of the cells of the immunized, just as a key fits a lock, then the cells are stimulated to produce excessive numbers of receptors, and these constitute the anti, or immune body. It is possible to produce from rennet, egg-albumin, cow's milk, and from many other albuminous substances, immune bodies by injecting these substances into animals. (Figs. 14, 15.)

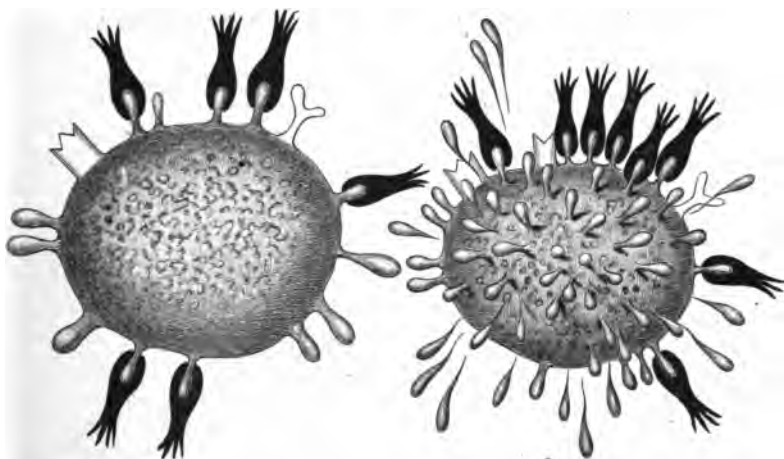


FIG. 14.—EHRlich'S LATERAL CHAIN THEORY. Cell with numerous receptors of various kinds and shapes to which are united the toxin molecule. Note the free receptors.

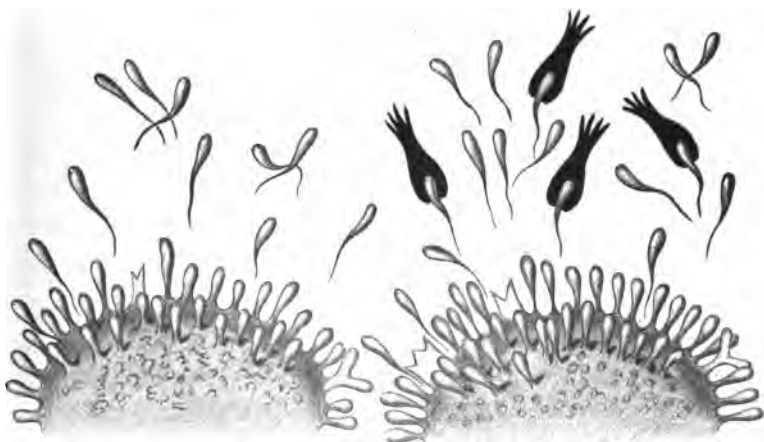


FIG. 15.—EHRlich'S LATERAL CHAIN THEORY. In one figure the free receptors (anti-bodies) are united with the toxin molecule, the attached receptors have no haptophores united to cell.

IMMUNITY.

List of immune bodies and their anti-bodies (Ricketts).

Antigens or Immunizing Substances	Products of Immunization		
<i>Toxins</i>	<i>Anti-toxins</i>		
<i>Complements</i>	<i>Anti-comple- ments</i>		
<i>Ferments</i>	<i>Anti-ferments</i>		
<i>Precipitogenous Substances</i>	<i>Precipitins</i>		
<i>Agglutinogenous Substances</i>	<i>Agglutinins</i>		
<i>Opsinogenous Substances</i>	<i>Opsinins</i>		
<i>Cytotoxin Produc- ing Substances</i>	<i>Cytotoxins. . .</i> <table border="0"> <tr> <td> <i>Hemolysins</i> <i>Bacteriolysins</i> <i>Special cytotoxins</i> <i>Such as</i> <i>Spermatotoxin</i> <i>Nephrotoxin</i> <i>Hepatotoxin, etc.</i> </td> <td> <i>Consisting of two bodies Complement Amboceptor</i> </td> </tr> </table>	<i>Hemolysins</i> <i>Bacteriolysins</i> <i>Special cytotoxins</i> <i>Such as</i> <i>Spermatotoxin</i> <i>Nephrotoxin</i> <i>Hepatotoxin, etc.</i>	<i>Consisting of two bodies Complement Amboceptor</i>
<i>Hemolysins</i> <i>Bacteriolysins</i> <i>Special cytotoxins</i> <i>Such as</i> <i>Spermatotoxin</i> <i>Nephrotoxin</i> <i>Hepatotoxin, etc.</i>	<i>Consisting of two bodies Complement Amboceptor</i>		

Synonyms.

Complement.
Alexin.
Cytase.

Amboceptor.
Immunkörper.
Zwischenkörper.
Intermediary body.
Fixater.
Preparater.
Copula.
Desmon.
Sensibilisatrice substance.

It is well known that rennet coagulates milk, but if some of the serum of an animal immunized against rennet is added to the milk, the latter cannot be coagulated because the anti-rennin combines with the rennet and renders it inert.

The production of bacteriolysins is explained by Ehrlich's lateral-chain hypothesis. Immunization against bacteria which do not pro-

duce soluble toxins is easily secured by repeated injection of either dead or living bacteria into the organism. It is not easy, however, to confer passive immunity, as in the case of diphtheria, by the injection of the serum of the immunized animal. The immune body is alone present in the serum generally and some complement must be added to effect bacteriolysis. The serums which aid in the solution of bacteria are known as anti-bacterial serums, which, though not anti-toxic, may check invasions and aid in recovery by destroying bacteria. It is possible to effect an *in corpore* bacteriolysis in the case of typhoid fever if the immune body and complement are injected in sufficient amounts and proportions. As yet the results are not satisfactory from a clinical standpoint.

Cholera and typhoid organisms do not produce soluble toxins in the body, but when they are disintegrated therein, soluble poisons (intracellular) are liberated.

Bacteria may become accustomed to the fluids of the body by a similar process and may elaborate free receptors for their own protection, i.e., anti-bacteriolysins. (Welch's theory.)

In the aged, and in chronic disease of the liver and kidneys, the complement existing in the blood may become reduced in quantity, and the individual may succumb to an infection, which ordinarily would be mild.

ANTI-TOXINS, VACCINES, AND TOXINS.

The following is Wassermann's list of anti-toxins:

Anti-toxins for bacterial toxins:—

Diphtheria

Tetanus

Botulism

Pyocyaneus

Symptomatic Anthrax

Anti-leucocidin, an anti-toxin against the leucolytic poison of staphylococcus

Anti-toxins for the blood dissolving toxins of certain bacteria.

Anti-toxin for animal toxins:—

Anti-venene for snake venom

Anti-toxin for spider poison

Anti-toxin for scorpion poison

Anti-toxins for certain poisons in fish, eel, salamander, turtle, and wasp sera.

Anti-toxins for plant poisons:—

Anti-ricin for castor-oil poison

Anti-abrin for jequerity bean poison

Anti-robin for locust bean poison

Anti-croti for croton-oil bean poison

Anti-pollin for pollen of plants that produce hay-fever.

Manufacture of Anti-toxins.—If small doses of a given poison, such as diphtheria toxin, be repeatedly injected into a susceptible animal, and if the dose is gradually increased, there appears, after a time, in the blood serum, an anti-body, or anti-toxin. This substance in the serum is secreted by the cells and corresponds to the free receptors in Ehrlich's lateral-chain theory. If an animal is injected with the anti-toxin, and then with a large dose of toxin—say ten times the amount necessary to kill it if it had not received the anti-toxin—it will not be harmed. Here the free receptors artificially supplied to the animal unite with the haptophorous chains in the toxin molecule, and neutralize, or bind, the toxophorous or poisonous chains in the molecule, and prevent toxophore from attacking important vital cells belonging to the animal. But if the anti-toxin and toxin, after being mixed in a test-tube, are injected into a susceptible animal, no harm results, if they are in proper proportions, since the same thing has happened *in vitrio* that happened in the animal, the receptors and haptophores have united; the toxophores are bound, and the animal is unharmed.

The manner of making the *diphtheria anti-toxin* can be taken as a type.

Diphtheria bacilli are grown for several weeks in bouillon at 37° C.; as the bacilli grow they elaborate a very powerful poison or toxin, which is highly complex in composition. It is easily decomposed by heat, light and oxygen, and should be used soon after it is prepared. After the cultures have grown for several weeks, 5 percent carbolic acid, or tri-cresol, is added, and the bouillon is filtered through a porcelain or Berkefeld filter, and is then stored in sterile bottles in an ice chest. Horses are generally immunized, since they are susceptible to the action to the toxin, and are easily managed. Before being used they are carefully tested with tuberculin for tuberculosis and with mallein for glanders. Being very susceptible to infection with tetanus while undergoing treatment, a prophylactic injection of *tetanus anti-toxin* is given each animal. McFarland found that the death rate from tetanus, in a large stable, was greatly reduced after using tetanus anti-toxin as a prophylactic measure.

To make anti-toxin, a very virulent toxin is employed. A horse, previously examined for health, is injected with from $\frac{1}{2}$ to 1 c.c. of toxin. This is followed by a rise of temperature, local reaction, and systemic disturbance. After waiting for all reactions to disappear a second injection is given, which is followed by others larger in size, until, after a few weeks or months, 1,000 c.c. of toxin are injected at one time (enough to have killed a dozen horses that had not received the smaller doses previously). The injection of the toxin is followed by an immediate fall in the anti-toxic power of the serum, only to be followed by a quick rise. The horse will not produce anti-toxin indefinitely. After the animal has been immunized sufficiently, his blood is drawn from the jugular vein, and after the clot has formed the serum is drawn off and stored.

McFarland found that a horse was capable of producing enough anti-toxin to protect 806 other horses against doses of toxin, each one of which was equivalent to the total amount of toxin that the immunized horse received. Thus there is evidently a tremendous overproduction of anti-toxin far above the needs of the animal.

The various component parts of the toxin stimulate the cells of

the horse to produce the receptors, or anti-toxin. The toxoids, themselves not poisonous, have the property of stimulating the production of anti-toxin. We measure the anti-toxic powers of the anti-toxin with units arbitrarily devised. *An anti-toxic unit is ten times the least amount of anti-toxic serum that will protect a guinea pig weighing 300 grams (standard) against ten times the least certainly fatal dose of diphtheria toxin.*

To standardize anti-toxin, we must employ animals, into the bodies of which toxins and anti-toxins are injected. If a certain amount of anti-toxin is necessary to protect a guinea pig against ten times the minimum fatal dose of toxin per 100 grams of guinea pig weight, then we know that the anti-toxin contains so many units. *A unit dose of toxin is the smallest amount of toxin necessary to kill a guinea pig weighing 300 grams, or the dose per 100 grams of guinea pig necessary to kill.*

Ehrlich's method of standardizing is to obtain an anti-toxin of known strength (anti-toxins do not deteriorate or vary as do toxins). A standard anti-toxin made by Ehrlich is now everywhere used, and is furnished by him from his institute.

Against this standard anti-toxin a toxin of unknown strength is measured by means of guinea pigs. The toxin unit thus found is then used to determine the anti-toxic unit of anti-toxins of unknown power.

The power of anti-toxic sera varies; some contain from 200 to 300 units only per c.c.; others may contain even 1,700 or 2,000 per c.c.

Anti-toxic serum is preserved by the addition of .5 percent of tri-cresol or phenol. It remains practically unchanged in strength for a year or more. When used it is common to inject from 2,000 to 5,000 units.

It is not only of value as a curative agent, neutralizing the toxins already formed, but is valuable as an immunizing one against infection. If injected early in a case of diphtheria, it is much more likely to do good, than if used later. Some desperate cases have received 100,000 units and have recovered.

Tetanus Anti-toxin.—Tetanus anti-toxin is produced in a manner similar to that of diphtheria anti-toxin. As the horse is exceedingly sensitive to tetanus toxin, before the immunizing process is begun, the toxin is attenuated by heat or iodine.

The anti-toxin is standardized, as in diphtheria, by testing its potency against the toxin. A guinea pig of 500 grams weight is used, and test toxin is employed of such strength that .01 c.c. will kill this guinea pig in about four days. This amount of toxin is neutralized by $\frac{1}{1000}$ of a unit of anti-toxin, or one unit of anti-toxin will protect 1,000 guinea pigs against the minimum fatal dose of tetanus toxin.

There can be no doubt that tetanus anti-toxin, if given with the toxin or soon afterwards, is a potent means of preventing lethal action of the toxin. Tetanus toxin enters into such quick combination with the cells of the motor elements of the nervous system, and the union is so permanent that it is difficult for the anti-toxin to form any union with the combined toxin. If an immune animal whose blood is powerfully anti-toxic received into his central nervous system a dose of toxin he will succumb at once, the anti-toxin apparently has an inferior valency, or combining power. If, however, it meets the toxin before it reaches the nervous system, it, by its receptors, binds the haptophores, and this prevents any combination of the toxophores with the receptors of the nervous system cells.

In general, anti-toxin is ineffectual if administered when acute toxic manifestations of the disease are in evidence. It has been found by Calmette and McFarland that if dried tetanus anti-toxin is sprinkled over wounds infected with tetanus bacilli, or impregnated with toxin, that it acts in a very prompt and effectual anti-dotal way.

If the toxic symptoms appear shortly after the infecting wound is received it is well known that the prognosis is extremely grave. In such cases, and in those that come to the care of the physician late and after the toxic symptoms have appeared, the anti-toxin must be used in large amounts directly about the wound to neutralize the uncombined toxin into the general circulation and directly into the nervous tissues or into the ventricle of the brain, in the hope that

the excess of free receptors of the anti-toxin may engage the haptophores and toxophores of the toxin molecule already attached to the receptors of the nervous cells in the floor of the fourth ventricle.

Streptococcus Anti-toxin.—While there are at least several *strains* of streptococci, it is a fact that the toxins produced by all have the same characteristics and properties. The toxin is of the nature of a diastase, which is destroyed by a temperature of 70° C. In addition to the ferment of a diastatic nature others of hæmolytic power are formed. This is called streptocolysin and it is said by Reudiger to possess both haptophorous and toxophorous chains in the toxin molecule. It is also destroyed at 70° C. Jaundice and petechial rash is often found in streptococcic infections. It causes a blood stained œdema and exudate at the site of infection in rabbits killed by the injection.

The anti-toxin is prepared by injecting horses with living cultures of very virulent streptococci, beginning with small doses and increasing them gradually. The last dose administered in the immunizing process may be 600 c.c. of a virulent culture. Four weeks after the last dose is given the serum is withdrawn. It is thought by Marmorek that the action of the serum is anti-bacterial, rather than anti-toxic. It has been found that the use of streptococci from human sources is the most efficient for the immunization of horses.

Anti-streptococcus serum is of some value in infection and diseases caused by streptococci. Of these it has been used in puerperal fever, erysipelas, and septicæmia. It has by no means won an undisputed place, like diphtheria anti-toxin.

The **Anti-pneumococcus** serum is prepared in the same way. Horses are immunized by the injection of living cultures, and the horse's blood, after a period of treatment by cultures, is drawn off, preserved with tri-cresol and used in a manner similar to diphtheria anti-toxin. Its use has not been attended with any marked results. It is a curious phenomenon that pneumococci grow better in the serum of a horse immunized against pneumococci than in normal horse serum.

There are anti-toxic sera for use against **Botulism**, or **meat pois-**

oning, pyocyaneus infection, hay-fever, staphylococcus infection, Malta fever, and typhoid, that have been used without much success. They have a certain scientific interest, but are of no great clinical value.

Anti-plague Serum.—Yersin, a French bacteriologist, treats horses with living cultures of plague bacilli, and after a long period of immunization uses a serum which either effectually vaccinates an individual against the plague, or greatly modifies the disease after it has once begun. Later it was found that heat killed cultures were just as effectual.

In 142 cases of plague treated with the serum, 24 died, a mortality of 14.78 percent, while in 72 cases untreated, 46 died, a mortality rate of 63.72 percent.

The action of the serum is bactericidal, as well as anti-toxic. The dose varies with the stage of the disease, 5 c.c. is an effective prophylactic dose, while from 20 to 300 c.c. have been used often as curative doses.

VACCINATION.

By the use of attenuated, or killed micro-organisms, it is possible to effectively vaccinate men and animals against many diseases. Notably, small-pox, hydrophobia, plague, cholera, typhoid fever, anthrax and quarter-evil.

Vaccination Against Small-pox.

There is now no doubt that vaccinia or cow-pox is but modified small-pox in the cow. The causal agent of small-pox, through its life in the tissues of the cow, becomes so modified that it does not produce in man variola, but vaccinia. This causal agent is believed to be a protozoan, called by its discoverers *Cytoryctes Variolæ*.

By the term vaccination, in its strict sense, we mean the application of attenuated small-pox virus, weakened by passage through kine, to human beings and infecting them with the modified disease. The

disease is localized at first at the site of inoculation, and a bleb or vesicle forms. As a rule the disease does not become generalized. It creates, in the vaccinated individual, an active immunity against small-pox. The toxins diffused through the blood-stream stimulate the cells of the body into forming either anti-toxic bodies, or anti-body substances. These correspond to the amboceptors, according to Ehrlich's theory, and are capable of either aiding the dissolution (lysis) of the infecting agent by acting as the immune body in conjunction with the complement, or as free receptors in neutralization of the toxins.

These various substances, as yet unknown, remain for a long period within the body of the vaccinated person and may protect it for years against invasion and infection with the cytocytes in virulent form. A person who has variola cannot be vaccinated, subsequently he is immunized against vaccinia by this attack of variola, just as he can be immunized against variola by vaccinia infection.

Since Jenner first discovered that cow-pox introduced into the body prevented small-pox, it has been the world-wide custom to use either the dried virus or liquid glycerinized virus from the cow or human beings in the process of vaccination. It has been found that human virus generally used was likely in rare instances to transmit syphilis, so it is now the universal custom to use cow virus. This virus is collected from fresh vesicles in calves or young heifers, as clean as possible, as it is used as seed to inoculate the animals and the operation is done under strict anti-septic precaution. After a week the virus is collected under similar anti-septic precautions by scraping the base of the vesicle with a sterile curette. The pulpy substance thus obtained is mixed with glycerine and stored for a month or more. The action of the glycerine is to rid the virus of many of the bacteria, through, it is supposed, a hydrolytic action. This virus is then rubbed into the skin of the individual to be vaccinated under strict aseptic precautions. At the end of a week, a pearly white vesicle is formed, and it is then considered that vaccination has "taken" and that the individual is protected against

variola. This action of immunization is supposed to be complete on the fourth day after the virus has been introduced. This is a matter that is difficult to decide, but the immunization process is, no doubt, a very slow one, like every other immunizing process where the immunity is autogenous and active, and not passive, as in the case of diphtheria anti-toxin.

There can be no doubt about this being one of the greatest boons that mankind has ever received. Vaccination is attended with some risk. Septic infection with streptococci sometimes follows, likewise tetanus infection. In both instances this may be due to the contamination of the vesicle on the calf before the virus is lifted.

Vaccination Against Cholera.

By the injection of the bodies of dead bacteria, or attenuated live ones, especially those containing in their cells insoluble poisons, it is possible to create in the animals experimented upon a powerful active immunity against the action of living virulent bacteria of the same species.

By the attenuations of cholera spirilla, Haffkine has produced vaccines which effectively protect individuals against infection with cholera, or if they become infected with the disease, it is so modified that they can, and do, more easily recover. He employs two vaccines, a weak one and a stronger one. The weak one is used to protect against the stronger one, which is the effective vaccine.

The weak, or first virus, is prepared by growing the cholera vibrios at a high temperature, 39° C., in a current of air. The stronger is prepared by passing the vibrios through a series of guinea pigs, so increasing the virulence that the virus is invariably fatal to the guinea pigs in eight hours. The best method is to use a culture that kills a guinea pig in twenty-four hours by peritoneal injection. After the animal is dead, the peritoneal exudate is collected, and grown at 35° C., the most favorable temperature for the organism to multiply. This exudate is injected into a second guinea pig, and its exudate is,

after incubation, injected into guinea pig number three, and the process is done repeatedly until the virulent virus that is lethal in eight hours is obtained. This is called *virus fixe*. After cultivating this virus on agar, the surface growth is washed off with sterile water (8 c.c.) and $\frac{1}{8}$ part of this is used as a dose. As the virus rapidly attenuates it must be reactivated by passing it through guinea pigs from time to time.

The first injection is given in the flank, and the second follows in five days. Accordingly as the symptoms are severe, so will the resulting protection be strong. Haffkine has given 70,000 injections without an accident. The following results were obtained by Haffkine who worked in India for the British Government:—

POPULATION.	CASES—CHOLERA.		DEATHS.	
	Total	Percent.	Total	Percent.
Non-inoculated, 1,735 - - - -	171	10.63	113	6.51
Inoculated, 500 - - - - -	21	4.2	19	3.8

The immunity conferred by this mode of vaccination is not complete until ten days after treatment.

Vaccination Against Typhoid.

By the injection of sterilized cultures of typhoid bacilli, it is possible to create an immunity of a moderate kind against enteric fever. The method has been perfected by Wright, and his mode of procedure is to secure a virulent culture of typhoid, which is tested on guinea pigs, and the minimum lethal dose for a 100 gram guinea pig is used as the dose for man. This dose varies from .5 c.c. to 1.5 c.c. of an old culture sterilized by heat at 60° C., and preserved with lysol. After the injection there is often redness and pain at the site of inoculation, some fever and lymphangitis. The results obtained in vaccinating the troops in South Africa are marked. Of the garri-

son of Ladysmith comprising nearly 12,000 troops, 1,705 were inoculated; 2 percent contracted typhoid afterwards, and 4 percent of these died of the disease. Among the non-inoculated, numbering 10,529, 14 percent contracted typhoid, and 3.12 percent of 10,529 died of the disease. It seems that this form of vaccination, in a great measure, prevents the infection with typhoid, and modifies the disease after infection occurs.

Vaccination Against Plague.

Haffkine, in India, has vaccinated many natives and others against plague by somewhat the same methods employed in anti-cholera vaccination. The *B. Pestis* is cultivated in flasks of bouillon; as it grows, the stalactite-like scum on top is shaken from time to time to the bottom of the flask. After growing for six weeks in the bouillon, the culture is killed at 70° C. for three hours. It is then used as vaccine, 3 c.c. is the usual dose for man, 2 c.c. for woman, and children still less. After the inoculation, heat and redness appear at the site of inoculation, and the patient feels ill and has some fever. Haffkine holds that immunity against the plague is complete in twenty-four hours after vaccination. His results are at times really very good. In a village, Unhera, among 64 uninoculated people, there were 27 cases with 26 deaths. Among 71 inoculated persons under the same conditions, and of the same families as the uninoculated, there were 8 cases and 3 deaths. The fatalities among the unvaccinated exceeded those among the inoculated by 89.65 percent.

The Indian Plague Commission reported that the measure was valuable as a means of preventing infection; while it was not an absolutely certain means, yet it sensibly diminished the death rate.

Vaccination Against Anthrax.

Of all forms of vaccination against disease with attenuated bacteria this is the most successful. Its use is confined to

domestic animals, sheep, cattle, and horses, and has reduced the mortality in the country where it is used from 10 percent to .5 percent. The method requires the employment of two vaccines made of attenuated anthrax bacilli. No. 1 is a culture of bacilli attenuated by growing them at a high temperature, 42.5° C., in a current of air for twenty-four days. No 2 is grown at the same temperature for only twelve days. The first vaccine is used to immunize the animal against the second, which causes a marked local reaction, and which the real immunization agent against infection with virulent anthrax bacilli. The injections are given about one week apart. Many State Governments as well as the Federal Government of the United States supply the vaccine gratis to stock raisers and others.

Vaccination Against Black-leg or Quarter-evil.

Quarter-evil, or Rauschbrand, is due to a specific bacillus. Vaccination against this disease may be accomplished by inoculating with a powder consisting of dried muscle from the affected part of infected animal. There are two vaccines, No 1, and No 2. The first is prepared by heating (and thus attenuating) the bacilli up to 103° C. The second is prepared by raising the temperature up to 93° C. These vaccines are given at a short time apart, and the immunity is effective. The method is valuable to stockmen.

Vaccination Against Tuberculosis.

It is possible to vaccinate animals against tuberculosis by the use of attenuated tubercle bacilli. To accomplish this, the sole requisite is to so weaken the bacilli used to immunize, that there is not any likelihood of causing any lesion. By long cultivation on culture media bacilli are so attenuated that they cannot cause harm to a guinea pig, even if repeatedly injected. Guinea pigs may be able to withstand inoculation with virulent bovine bacilli; cows may be immunized against bovine bacilli by inoculating them with weak

human cultures. Koch's new tuberculin, made by grinding to a powder the dried bodies of tubercle bacilli, is also able to set up an immunity in animals, and to a limited extent in man. It is used in several large sanitariums devoted to the cure of tuberculosis, as a therapeutic agent. Those using it claim that it immunizes the individual and thus increases his resisting powers.

The Tuberculins.

The toxin of the tubercle bacilli (tuberculin) is prepared by growing the organism for a long period in glycerinized veal broth, after which the flasks are steamed in a sterilizer for an hour or more, and then the bacilli are filtered out through porcelain filters. The filtrate is reduced by boiling to $\frac{1}{10}$ of its bulk, and to this a half of one percent of carbolic acid is added as a preservative. If this toxin, even in minute doses, is injected under the skin of a tuberculous animal, it acts as a powerful poison. In a few hours, it causes a rapid rise of body temperature, accompanied by nausea and, perhaps, vomiting. About the localized foci of tuberculosis, a vigorous reaction occurs. Around indolent old sores and other lesions there is a tendency to heal by the casting off of necrosed tissues, and the infiltration of the peritubercular area with leucocytes. In lupus (tuberculosis of the skin) redness and heat occur about the lesion. This febrile phenomenon following the injection of tuberculin into tuberculous animals is a valuable diagnostic feature towards the recognition of tuberculosis in animals and in man. In 90 percent of cases the reaction is trustworthy.

Tuberculin acts as a fever producer in an unknown way. It is supposed, however, that the intense local reaction produces fever through active tissue changes.

Its use in man has been much questioned, as it is thought by some to disseminate the disease from original and confined foci. This however has been denied. Many able clinicians use it and recommend it. (Osler, Trudeau, Musser.)

Koch's new, or T.R. tuberculin was, like the old, designed by him as a therapeutic agent for the cure of tuberculosis. It is made by pulverizing the bodies of living tubercle bacilli and dissolving the residuum in an indifferent fluid. It produces a more intense reaction than the old tuberculin. Like the old, it is used in the treatment of lung, bone, laryngeal, and skin tuberculosis. It certainly causes a local reaction about tubercular foci, and no doubt aids in the formation of an active immunity to the disease.

The dose of tuberculin varies from $\frac{1}{10}$ of a milligram to 5 mgs. and in case the first dose does not produce a reaction, it should be repeated.

Mallein.

Mallein is a preparation made from the toxin of the glanders bacilli, and is prepared precisely as the old tuberculin. By increasing the virulence of the glanders bacilli, by passage through a series of guinea pigs, a highly virulent bacillus is obtained. It is then grown in glycerinized bouillon for a month at 37° C. The resulting fluid is sterilized by heat and filtered through a Pasteur filter. The filtrate is evaporated to half its quantity, and to this a small amount of carbolic acid is added in order to preserve it. Of the mallein thus prepared 1 c.c. should kill a rabbit in one to two weeks.

In a horse with glanders, the injection of mallein is followed by a large painful swelling at the injective site, with this there is a rise of temperature, which is the diagnostic reaction that indicates infection with glanders. In this respect the reaction is like tuberculin. In healthy horses no rise of temperature follows the injection, and the resulting swelling more quickly subsides. Mallein has been used as a prophylactic agent against glanders with some success.

Immunization Against Hydrophobia.

While the actual causal agent of hydrophobia has thus far eluded bacteriologists, certain well marked histologic lesions have been

discovered in the ganglia of the central nervous system, and in the medulla, which are not found in any other disease. This dispels all doubt as to the fact that hydrophobia is a real clinical entity.

It is possible to immunize animals and man against this disease, by the use of attenuated virus. In common with many other viruses, that of hydrophobia can be weakened through the action of either heat, drying, light, or chemicals. Pasteur found that by drying the spinal cords of rabid animals for two weeks, they become totally avirulent. If the cord is dried but three or four days, the virulence is but slightly modified. Immunity to rabies can be produced by injecting minute quantities of the poison, and then gradually increasing the dose until virulent virus can be employed.

Modification of the amount of poison used may be affected by employing equal quantities of spinal cords from rabid animals that have dried varying lengths of time. The vaccine consists of pieces of cord, 1 cm. in length, from rabbits that have been killed by inoculation with fixed virus. This is emulsified with sterile salt solution. Cord that has dried for fourteen days is first injected, after which cords that have dried fewer and fewer days, until, finally, one that has dried only three days is injected.

In cases of bites by rabid dogs on the face or head, the vaccination must be rapid, so two injections per diem are given. In Berlin the weakest injection used (the first) is made from a cord that has dried but eight days, and the course is much quicker. The effect of this mode of inoculation is to produce in the bitten individual a very rapid active immunity, quicker in its action than the infection. The treatment is solely prophylactic and in no way curative. If symptoms of rabies have set in, the treatment is of no avail. In rabies the incubative period is about six weeks, so that there is plenty of time to immunize the patient by injection with attenuated virus.

Since the immunizing process is always begun *after* the bite of a rabid, or supposedly rabid dog, it differs from other vaccinations, which are resorted to *before* infection.

Results of Treatment.

In rabies the total mortality before the introduction of vaccination was not less than 10 percent. Among the same class of patients in the Pasteur institutes, the death rate of all cases, early and late, has been reduced to a fraction of 1 percent. Those cases in which the bites are on the head, are always more serious, and the mortality is higher. Like tetanus the virus travels, it is supposed, from the site of injury to the central nervous system by way of the nerves. If the bite was on the toe, it would take longer for infection to reach the brain, than if it was on the upper lip. This is a very plausible explanation of the varying incubative periods in both tetanus and hydrophobia.

Coley's Fluid in the Treatment of Tumors.

This method of treatment is in no wise a prophylactic one, but strictly a curative one. It consists in the injection of the toxins of streptococci, in the hope that they will cause a shrinking, or disappearance of malignant sarcomata. An attack of erysipelas (it has long been observed) occurring in a patient with some malignant disease, has the effect of causing a disappearance, or retrogression, of the tumors. Artificial infection with streptococci was then practiced with the idea that it might produce the same effect. But this was found to be dangerous. Coley prepared toxins of streptococci by allowing them to grow with the *B. Prodigiosus*. The mixture after a long period of incubation was sterilized by heat, and the fluid thus obtained was injected into the tissues. Virulent strains of streptococci are used and the dose of the dead culture is about half a drop given under strict anti-septic precautions. Out of 200 cases many were cured. In 35 cases treated by other surgeons 26 tumors disappeared, and 14 of these cases were alive from two to four years after. The best results are obtained in spindle cell sarcoma, and the poorest in the melanotic variety. The method by no means

should be employed where the tumor can be removed by operation. It cannot supplant the knife, and only in inoperable cases or as a supplementary treatment where other forms of treatment are employed, should it be used.

Opsonins and Opsonic Index.

Peculiar substances in blood serum have been called by Wright and Douglass *opsonins* (Greek: prepare food for). If fresh blood is mixed with an emulsion of some bacteria and then incubated for half an hour, it will then be found that many of the bacteria are within the polymorphonuclear leucocytes. If the serum is washed away from the leucocytes before adding bacteria, none of the latter will be found within the leucocytes. This proves that the serum has some influence on phagocytosis. In order to show that this effect is on the bacteria rather than on the leucocytes, the bacterial suspension may be treated with some serum for half an hour and then washed free from this serum by means of a salt solution in a centrifuge, and then mixed with some serum-free leucocytes, then it will be found that phagocytosis occurs as before. The bacteria have been "sensitized." According to Wright this action is comparable to cooking.

Phagocytosis then depends upon the action of some serum upon bacteria, which are coped with in the body, first by the action of the serum, and then by the leucocytes. This opsonic substance, like the amboceptors, sometimes disappears from the blood. It is thermostabile.

The quantitative action of phagocytosis may be estimated by Leishman's method. He mixed blood and an emulsion of bacteria in salt solution in equal quantities, and allowed them to stand for 30 minutes in the incubator. After this the mixture was stained and the average number of bacteria per leucocyte was obtained. The result was known as the *phagocytic index*.

Wright has devised the following technique. Young cultures, a few hours old, are employed. These are scraped off agar tubes and mixed with salt solution. After this has sedimented, the supernatant fluid is separated from the bacterial masses by a centrifuge; is pipetted off, and preserved.

Washed leucocytes are obtained by collecting 2 cc. of blood in 30 cc. of salt solution containing 1% citrate of soda to prevent blood coagulation. The serum and citrate of soda are separated from corpuscles by washing twice in a centrifuge. The upper layer of the sediment is rich in washed leucocytes, and is used in the experiments.

To obtain the opsonic index, blood serum from various cases is collected. In the case of staphylococcus infection—say carbuncle—the blood serum is drawn from the patient and, with equal portions of an emulsion of staphylococci (young culture), and a suspension of washed corpuscles, is

thoroughly mixed in a pipette, which, after the ends are sealed, is placed in an incubator for 15 minutes. A drop of the mixture is then spread upon a slide; fixed, and stained with Jenner's stain. The number of staphylococci in 50 polynuclear leucocytes is determined and divided by 50 to obtain the average.

At the same time that this experiment is being performed, some normal serum should be used in another experiment; an emulsion of staphylococci and washed leucocytes being used as above. After pursuing the same steps in this experiment as in the first, the average number of staphylococci per leucocyte is determined.

To obtain the opsonic index, it is necessary to know the ratio of staphylococci in the leucocytes heated with the carbuncular serum, and in the normal. If the normal serum leucocytes contained 10 staphylococci, and the carbuncular serum contained 15, the index would be 1.5.

In the case of tubercle bacilli, the latter must be heated to 100° C. to kill them, otherwise they will be agglutinated by the serum, and a homogeneous emulsion will not be obtained. After heating, the clumps must be broken up by grinding the masses in an agate mortar, adding a little salt solution from time to time until the mass is thoroughly broken up. The bacilli must then, after phagocytosis, be stained by carbol fuchsin and decolorized with acid alcohol. If the leucocytes are left too long in contact with the organisms they may become so engorged as to prevent counting, the number increasing from 5.7% after five minutes to 28.5% in two hours.

Highly immunized anti-bacterial serums have much greater opsonic powers than have normal ones, anti-streptococcus and anti-pneumococcus sera being especially powerful toward streptococci and pneumococci. It is possible to increase the opsonic powers of the blood of an individual suffering from an infection, by vaccinating him with killed cultures of the organism with which he was infected.

Wright has treated tubercular and septic infections in this way with excellent results, the opsonic index of the individual being very markedly raised.

CHAPTER V.

BACTERIA.

STREPTOCOCCUS PYOGENES.

Streptococcus Pyogenes.

Streptococcus Erysipelatus. Chain Coccus. (Fig. 16.)

Morphology and Stains.—Cocci grow in catenate form of from 4 to 40 individuals to a chain. Each coccus comprises two hemis-

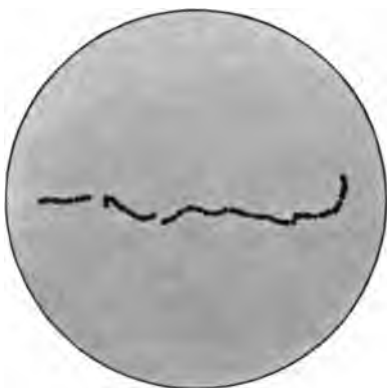


FIG. 16.—*Streptococcus pyogenes*. (Kolle and Wassermann.)

pheres divided transversely. Some chains appear branched, are not motile, and do not have spores. They can be stained with all basic stains, and are not decolorized by Gram's method.

Relation to Oxygen.—They grow either in the presence or absence of oxygen, and are, therefore, facultative aerobes.

Temperature and Food Requirements.

Develops best at 37° C. Will not grow at 47° C. Never vegetates luxuriantly on any culture media, and are most prolific on one that is faintly acid. On **gelatine** they grow scantily without liquefaction. The growth consists of discrete little masses, while on **agar** with glycerine, they appear translucent colonies of very small grayish granula. In **bouillon** cultures some varieties either cloud the medium uniformly, or else sedimentate in the form of little balls, the supernatant fluid remaining clear. It does not ferment sugars. In **milk** the growth is more luxuriant, and becoming acid, is totally *coagulated* in twenty-four hours. On **potato** the growth is invisible and scanty.

Vital Resistance.—Thermal death-point is 54° C. in five minutes. Virulence in dried albuminous matter (pus) is retained for months. If kept on ice, vitality and virulence are retained for months also.

Chemical Activities.—*Lactic acid* and *sulphuretted hydrogen* are produced, also *ferments* which have the property of dissolving fibrin under anaerobic conditions. They are also capable of dissolving red blood corpuscles, either in culture media or in the body. They produce a strong soluble toxin, which can be filtered from the bouillon and precipitated with alcohol. This causes necrosis, anæmia and death.

Habitat.—In *sewage*, *dwellings*, *dust*, on the healthy human body, and in the cavities of the respiratory tract, vagina, rectum, and in the feces. It is the cause of many diseases, i.e., erysipelas, puerperal fever, meningitis, pneumonia, endocarditis, peritonitis, tonsillitis, osteomyelitis, and the diarrhoea of children.

In general septicæmia streptococcus is found in the blood, and plays an important rôle in secondary infection, causing an aggravation of the original infection, and often death. It is especially active in phthisis, scarlatina, small-pox, and diphtheria, in which diseases

it is often the cause of death. Many of the symptoms of phthisis are due to the toxins of the streptococcus; cavity formation and hectic fever for example. Its virulence can be intensified by passing it through a series of animals, until, finally, $\frac{1}{1000}$ of a cubic millimeter killed in one day all the mice injected with this dose. The toxin contains a peculiar hæmolytic substance, which, as before remarked, dissolves red cells of the blood, hence the anæmia in septicæmia and in suppuration. The toxin of the streptococcus, if injected under the skin, causes redness like erysipelas. Coley's fluid containing this toxin is used to treat sarcomata, since infection with the streptococcus has been known to cause a disappearance of these tumors.

Agglutinations.—The serum from an animal injected with streptococci, or immunized against it, will agglutinate streptococci.

Anti-toxic sera have been prepared by injecting horses with highly virulent living culture of streptococci. The serum protects to a limited degree, and has some curative properties. Cultures of cocci from human sources have been found to produce the best toxins.

PNEUMOCOCCUS.

Streptococcus lanceolatus, commonly known as the **pneumococcus**, or **diplococcus lanceolatus**. (Fig. 17.)

Morphology and Stains.—This organism is usually found in the tissues and sputum, in the form of lance-shaped cocci, surrounded by a capsule. Is almost always associated in pairs, though sometimes in chains of five or six numbers. In albuminous fluids, or blood serum, and in milk, the organism exhibits a well defined capsule; in bouillon and other media, it loses the capsule and the lanceolate shape, and often appears spherical, in pairs, or chains. It is not motile, has no flagella or spores, is easily stained by all the basic aniline dyes, and keeps its color by Gram's method. Under certain conditions it strongly resembles the streptococcus pyogenes, and may be differentiated therefrom by growing it on agar smeared

with blood. The streptococcus causes a hæmolysis of the corpuscles, while the pneumococcus does not.

Oxygen Relations.—It is a facultative aerobe.

Grows rapidly, but never luxuriantly at 37.5° C.; at 22° C. much more slowly, often not at all.

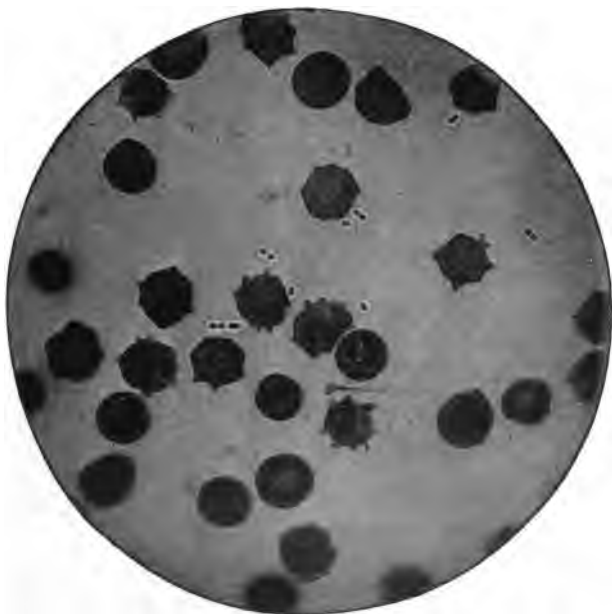


FIG. 17.—*Diplococcus pneumoniae*, from the heart's blood of a rabbit. $\times 1000$
(Fränkel and Pfeiffer.)

Vital Resistance.—Easily killed at a temperature of 52° C., exposed for ten minutes. Direct sunlight also kills it in twelve hours. While it quickly dies on ordinary culture media, it may live in dried sputum or pus exposed to diffuse sunlight and desiccation, for four months.

Cultures.—On **gelatine plate** it produces very minute colonies after quite a length of time. On **glycerine agar** it grows better, but the colonies are small and difficult to see. In both, the colonies are whitish, with a pearly lustre. On **blood serum** it grows in transparent colonies, slowly liquefying the media. In **bouillon** it grows feebly, with a whitish sediment, and in the form of chains. Here the growth is inhibited by the products of its own metabolism, i.e., lactic acid. If this is neutralized by putting chalk into the bouillon the growth becomes luxuriant and the bouillon becomes thick. On **potato** it will not grow.

Habitat.—Outside the human body it has not been found, but is normally present in the mouth of about 30 percent of all people. Even the alveoli of the lungs in health contain them. Human saliva injected into animals often causes pneumococcic septicæmia. They are also found on the conjunctiva and nose in health.

Chemical Activities.—No soluble toxin has been discovered. The toxic properties are due to an endo-toxin. This organism is a pyogenic one, and causes dense fibrinous exudates on serous membranes. All tissues of the body may be attacked. Some strains of pneumococci are more neurotoxic than others.

In rabbits an injection (intravenous) of pneumococci very often (33 percent) causes lobar pneumonia; certain strains cause lobular pneumonia habitually among the susceptible animals (Eyre). In human infection the organisms are forcibly inhaled into the deepest recesses of the lungs. Pneumonia may be hæmatogenous in origin also.

Besides pneumonia, any serous membrane may be attacked and pleuritis, peritonitis, pericarditis, or meningitis may be caused. Abscesses anywhere may be due to the pneumococcus. Mucous membranes of the throat often are affected, middle ear abscesses, also may be caused by this organism. Pneumococcic septicæmias are common.

During pneumonia, pneumococci may be recovered from the blood before the crisis by means of blood cultures; 10 c.c. of blood

abstracted from veins is mixed with 500 c.c. of milk and incubated. In twenty-four hours pneumococci, if present, grow luxuriantly. Just before the crisis the organisms will not grow.

Immunity and Susceptibility.—The susceptibility of man varies greatly. Exposure to cold and hardship of various kinds predisposes pneumonia. One attack does not prevent another. It has been observed that normal leucocytes only become phagocytic towards the pneumococcus when lying in anti-pneumococcic serum. It has even been noticed that these organisms grow better in the anti-serum, rather than in the normal serum. Animals have been immunized by injecting cultures and toxin. The immune serum thus produced protects small animals against infection, and stimulates phagocytosis. It has been used therapeutically in man for the cure of pneumonia with doubtful results.

Agglutination of pneumococci is caused by the blood of infected individuals, even diluted at 1-60. Immune serum also has the same action.

Opsonins.—Peculiar bodies found in anti-toxic sera (which are not anti-bacterial) stimulate polynuclear leucocytes and render the cocci more susceptible to phagocytosis (bacteriotropic action).

COCCUS OF MENINGITIS.

Streptococcus Intracellularis.

Diplococcus Intracellularis Meningitidis. (Fig. 18.)

Meningococcus.

This organism is thought to be the cause of cerebro-spinal meningitis. By some bacteriologists it is considered to be identical with the pneumococcus.

Morphology and Stains.—Resembles the gonococcus closely, because it grows in biscuit shaped pairs; is nearly always within pus cells; sometimes in the nuclei of the cells, and like the gonococcus it is decolorized by Gram's stain.

In reality it is a micrococcus, because it divides in two planes. It has no spores or flagella; is not motile; grows in short chains at times, and on ordinary media best at 37° C.

Relation to Oxygen.—It is an obligate aerobe.

Vital Resistance.—It is killed after 30 minutes' exposure to 65° C. and is easily destroyed by drying, and by light.

Cultures.—On **glycerine agar** it grows, sparingly, white viscid colonies; occasionally it develops on **potato**; thrives on **blood serum**, especially if smeared with blood, and does not liquefy the serum.

Habitat.—It is found in the pus from the meninges, sputum,

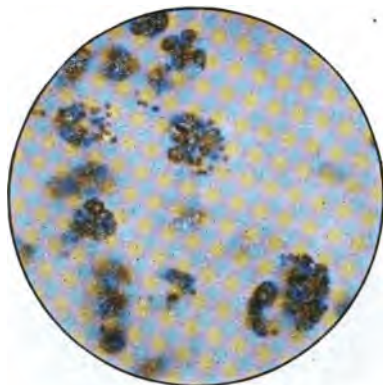


FIG. 18.—*Micrococcus intercellularis meningitidis*. (Kolle and Wassermann.)

urine and nasal mucus of persons afflicted with epidemic meningitis, or spotted fever. It has been found in the mucus membranes of healthy individuals and is the cause of epidemic meningitis. After spinal puncture, it may be seen in the pus cells, and the diagnosis of the disease is often made in this way.

Virulence.—It is scarcely virulent for lower animals. If given by hypodermics into the pleura, or peritoneum, it produces death in mice. Meningitis may be produced by sub-dural injection.

Chemical Activities.—Produces an endo-toxin but no soluble toxin. It is not chromogenic.

Agglutination is caused by immune serum.

STAPHYLOCOCCUS PYOGENES AUREUS.

Staphylococcus Pyogenes Aureus. (Fig. 19.)

Micrococcus Pyogenes.

Staphylococcus Pyogenes Aureus, Albus, and Citreus are known commonly as staphylococcus, or grape coccus.

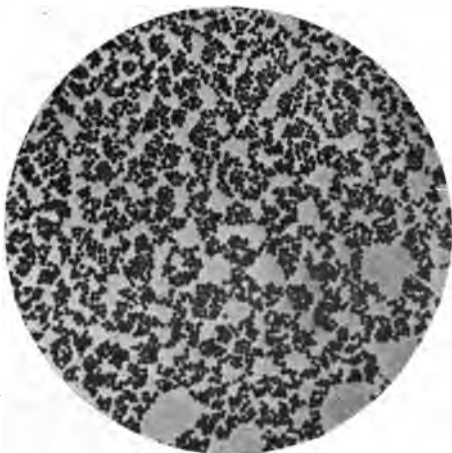


FIG. 19.—Staphylococcus aureus. (Williams.)

The *Micrococcus Pyogenes Aureus* only is here described.

Morphology and Stains.—Round cocci, often growing in bunches like grapes. Individual cocci dividing in planes. It stains very well with all basic dyes, and is not decolorized by Gram's method. It is not motile; has neither flagella nor spores.

Oxygen Requirements.—It grows well in oxygen, and poorly without it.

Temperature and Vital Resistance.—Thrives best at body temperature, but grows well at room temperature. Resists drying for over one hundred days in pus. Dry thermal death-point is 80°C . for one hour. Moist heat 70°C ., kills in ten to twenty minutes. Resists freezing temperature for many months.

Exceedingly resistant to formaldehyde, more so than some spore-bearing organisms. Resists light also.

Chemical Activities.—Produces a golden yellow pigment only under oxygen. Generates acids, but no free gases. Creates indol and sulphuretted hydrogen; ferments urea, and produces ferments that dissolve gelatine, and the coagulated proteids of milk. The toxin is soluble in water, and acts intensely, causing violent local reaction. If in the abdominal cavity, it causes peritonitis. Subcutaneously it may produce sterile abscess, or local necrosis.

Cultures.—In **gelatine** it rapidly forms golden yellow colonies, that quickly liquefy the gelatine. Sterile products of the growth also liquefy gelatine. On **gelatine plate**, yellowish to orange colonies are formed. On **agar streak** a luxuriant orange growth. In **bouillon** a marked even cloudiness, with fine pellicles on surface; sediment moderate, which upon shaking is broken up. **Milk** is rendered acid and curdles very soon, the curd being digested finally. (Fig. 20.)

Potato cultures are dry, whitish, then yellow, and finally deep orange.



FIG. 20.—Gelatine culture *staphylococcus aureus* one week old. (Williams.)

Habitat.—Widely distributed; found in dirty water, sewage, air, dust of streets and houses. Also deep in the layers of the skin. Normally present in the mouth, nose, rectum, anterior urethra, vagina, and external ears.

Pathogenesis.—In man it is the cause of carbuncles, abscesses, osteomyelitis, septicæmia, puerperal infection, and any inflammation of the serous membranes. It causes acne and boils; can, and does attack any tissue of the body. Endocarditis is a very grave affection that is caused by this organism. It also plays an important rôle in secondary infection, causing necrosis of previously infected tissues (tubercles) and is active in small-pox and diphtheria. Experimental endocarditis has been produced in animals by injecting it into the veins. By passage through animals it is rendered highly virulent. In young, diabetic and anæmic subjects, its action is often rapidly fatal. Its pathogenic action is often wide and disastrous. By growing it under anaerobic conditions its virulence may be intensified, and the activity with which it liquefies gelatine is an index of its malignancy.

In man acne, boils, and carbuncles have followed the rubbing of culture into the skin.

Immunity.—Thus far it has been impossible to produce any marked immunity either by anti-toxic sera, or by culture, living or dead.

GONOCOCCUS.

Micrococcus Gonorrhœa (Neisser).

Diplococcus Gonorrhœa, commonly called the gonococcus. (Fig. 21.)

Morphology and Stains.—The morphology of this organism is peculiar and characteristic. Always found in pairs which are cemented by an invisible substance. These pairs resemble coffee beans with the grooved sides opposite each other and slightly apart; or kidneys placed with the hilums facing each other.

In pus it is generally found within the protoplasm of the leucocytes,

about, though never within, the nuclei. It is non-motile; has no flagella, or spores, and stains readily with all the basic stains, but best with Löffler's blue. It is decolorized by Gram's stain. This point is most important in differentiating it from all other cocci, except the meningococcus. A diplococcus is said to exist normally in some urethras that resembles the gonococcus.

Oxygen Requirements.—It is a facultative anaerobe.

Vital Conditions.—It is cultivated with difficulty in culture media. Grows best at about 36° C. As it dies quickly in usual culture media, a special one must be employed; that containing blood and urine is best. It does not withstand high temperature, drying, or light, very long, and is very easily killed in culture by silver salts. In tissues of the urethra it may live many months.

Cultures.—On agar, containing ascites fluid, it grows very sparingly. The colonies are exceedingly delicate, and gray, turning to yellowish, and are scarcely above the culture media. It will not grow in **gelatine, milk, or ordinary bouillon**, but in one made of nutrose, serum, beef-extract, and peptone.

Habitat.—Never found outside the human organism, except on linen, towels, instruments, etc. It is in all senses a strict parasite.

Bacterial Activities.—Apparently does not produce a soluble toxin, but an endo-toxin (*gonotoxin*), which is highly resistant to heat.

Pathogenic Virulence.—This organism does not infect any of the lower animals. The "gonotoxin," if injected into small animals, produces a doughy infiltrated area, which undergoes necrosis. It has been found that filtrates of old cultures (sterile), if placed on urethral mucous membranes, can produce suppuration. In man, the organism causes a distressing disease (*gonorrhœa*), which may become a dangerous one, ending even in death. It may produce violent inflammation of the urethra, vagina, uterus, fallopian tubes,



FIG. 21.—Gonococci in pus. (Greene's Medical Diagnosis.)

and the peritoneum. It frequently affects the conjunctivæ of the newly born, and sometimes causes a pan-ophthalmia, which destroys the sight. It is a common cause of suppurating arthritis (gonorrhoeal rheumatism), endocarditis, pleuritis. In fact, any serous membranes may be infected, and very serious results follow. Cystitis caused by the gonococcus is sometimes followed by infection of the kidneys. In the urethra, the cocci may burrow deep beneath the epithelial cells, and set up a metaplasia, or abscess formation. The purulent exudate is rich in phagocytes gorged with cocci; often as many as 40 being found within a cell.

Immunity.—One infection does not confer immunity against further infection. There is no reliable means of producing artificial immunity.

MICROCOCCUS TETRAGENUS.

Micrococcus Tetragenus.

Morphology and Stains.—Round or oval cocci; found in pairs; more commonly in fours differing in size. In culture this form of growth is apt to vary, and not to be characteristic. In sections of human or animal tissues, tetrads only are found that are always surrounded by a capsule which is stained easily by eosin. The cocci are stained by Gram's method. It is not motile, and does not form spores.

Oxygen Requirements.—It grows very well in the presence of oxygen, and poorly without it.

Cultures.—Grows well on all common culture media. On **gelatine plates** its growth is characterized by small white colonies, elevated, with sharp outlines. It does not liquefy the gelatine. On **agar** it grows even more luxuriantly than on gelatine. In **bouillon** it thrives well, depositing a heavy precipitate. In **milk** it causes coagulation after four days. On **potato** it also grows, leaving a silvery streak where the inoculating needle was drawn.

Chemical Activities.—It produces acid in sugar bouillon, but does not form gas, indol, or H_2S .

Habitat.—Has never been found outside the human body; is normally present in the saliva, sputum of tuberculous subjects, in the cavities of phthisical lungs, and in abscesses.

Pathogenesis.—While causing a fatal septicæmia in mice, and abscesses in rabbits, it is not of much moment from a pathological standpoint, though it plays an important role in secondary infection in phthisis.

BACILLUS, MALTA FEVER.

Bacterium Melitensis.

Bacillus of Malta Fever.

Coccus of Malta Fever.

An organism belonging somewhere between the Coccacæ and Bacteriacæ. It is small, oval-shaped, and of about $.5\mu$ diameter. occurring in culture singly, in pairs, or in chains. In the latter form, the organism elongates and resembles, more strongly, bacilli. It is motile, and according to Gordon, possesses from one to four flagella, but it has no spores. Stains faintly with the common basic dyes, but not by Gram's method. It has been found in the blood during life, and by splenic puncture.

Cultures.—On **gelatine** its growth is slow, without liquefaction. On **agar** the growth, at $37^{\circ}C$., is more rapid. The colonies are pearly white, becoming yellow. In **bouillon** it produces turbidity, with a flocculent deposit. No pellicle is formed. On **potato** an invisible growth occurs. **Milk** is not coagulated, nor are acids or gases produced.

Pathogenesis.—It causes in man, Malta fever. Rabbits, guinea pigs, and mice are not susceptible to inoculation, but the disease can be produced in monkeys.

Agglutination.—The serum from an individual suffering from

Malta Fever agglutinates the bacilli, even in dilutions as high as 1-100.

Diagnosis of the disease can be effected by the agglutination test, and by splenic puncture, and blood cultures.

INFLUENZA BACILLUS.

Bacterium Influenzæ.

Influenza bacillus.

Morphology and Stains.—Very small short rods which are often in pairs, found within epithelial and pus cells, and in sputum; from 40 to 80 in a cell. Sometimes found chain-like. No flagella or spores are formed. Stains weakly. Carbol fuchsin, diluted, gives the best result. The ends of the bacilli stain more deeply than do the rest of the cells. It is decolorized by Gram's stain.

Oxygen Requirements.—It is a strict aerobe.

Cultures grow best on **blood smeared agar**, or in **blood bouillon** between 27° C. and 43°C.; best at 37° C. Blood or hæmoglobin is required for all cultures. In **bouillon** it grows in thin white flocculi. On **agar** in small transparent tiny columns, never luxuriantly. Grown in the same culture with staphylococcus aureus, it increases more luxuriantly (symbiosis). It is probable that the cocci, in some way, alter the blood of the culture media.

Vitality.—It is easily killed by light, heat and drying. Lives but a day in distilled water, and from eight to twenty-four hours in dried sputum.

Habitat.—Never outside the body; always a strict parasite. It is found in the mucous membranes of the upper respiratory tract, and in the mucous secretions.

Pathogenesis.—If pure cultures are placed on the mucous surfaces of monkeys, influenza results. Pure cultures injected into the peritoneum of guinea pigs cause fatal peritonitis. In man, it causes various affections of the upper respiratory tract—bronchitis, pneu-

monia, both croupous and catarrhal. Also conjunctivitis. It elaborates a powerful toxin, which produces strongly depressing effects on certain organs, especially nervous tissues. It is an important factor in abscess production in the middle ear, and elsewhere, and complicates many pneumonia cases, seriously interfering with recovery in young children, and the aged. Associated with the pneumococcus, its toxic effect is increased. It has been found in the blood, but no cultures have ever been made therefrom. In one



FIG. 22.—Pest Bacilli from spleen of rat. (Kolle and Wassermann.)

case of purulent meningitis, the influenza bacillus was the only organism found. No immunity results from infection. No leucocytosis occurs during infection. Serum from infected individuals agglutinates bacilli even if diluted 1-500.

PLAGUE BACILLUS.

Bacterium Pestis.

Plague Bacillus. (Fig. 22.)

Morphology and Stains.—Short plump rods with rounded ends, containing no spores, but probably flagella? Also surrounded by

capsule. Organisms from exudates, or blood, exhibit characteristically peculiar polar staining. They are often found within the leucocytes. In bouillon the organism grows in long chains; is stained with all the common basic dyes, but is not colored by Gram's method in cultures. It exhibits a great variety of involution forms when grown in salty culture media ($3\frac{1}{2}$ percent salt).

Relation to Oxygen.—Strict aerobes, the growth is stopped by the exclusion of oxygen.

Vital Requirements.—Grows well at 22° C., but best at 37° C.; is

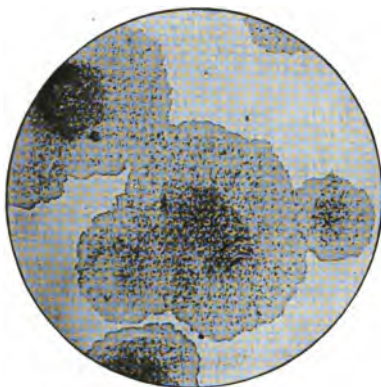


FIG. 23.—Colonies of plague bacilli forty-eight hours old. (Kolle and Wassermann.)

killed after a short exposure to 55° C.— 60° C., stands drying from four to eight days, and dies in water after a week. In the buried bodies of man and animals it lives from twenty-two to thirty-eight days. Withstands freezing for months, but does not stand light or chemicals very long.

Cultures.—Grows very well on culture media. In bouillon it thrives abundantly, with a heavy pellicle which produces dependent stalactites that drop to the bottom of the vessel. On gelatine

plates it grows in small elevated colonies, which are gray and transparent, and which do not liquefy the gelatine. (Fig. 23.) In **gelatine tubes** it forms a faint thread-like line, without liquefying the media. On **agar** the growth is whitish and abundant, and resembles the colon bacillus. Old cultures are luxuriant. **Milk** is not coagulated, and the growth is slight. **Potato** yields a slow whitish-yellow growth that is sharply outlined.

Chemical Activities.—Does not produce H_2S , enzyme, colors, or odors, but yields indol and nitrites. The toxin produced is not

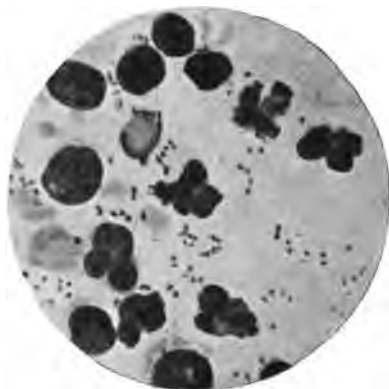


FIG. 24.—*B. Pestis* in pus of bubo. (Jackson.)

soluble and the filtrate is non-poisonous. Old killed bouillon cultures can be extracted and a highly poisonous substance precipitated therefrom with alcohol, or ammonium sulphate, that is lethal for mice.

Habitat.—Never found in healthy human bodies. In persons afflicted with plague, the organism is widely distributed in buboes and in the cutaneous pustules, lymphatics, and in the lungs in plague pneumonia; more rarely in the blood and other organs. In **animals**, plague occurs in rats. It is supposed that some tropical soil

bacilli infect rats, and becoming accustomed to the rodent's body, are eventually transmitted to man.

Pathogenesis.—Highly pathogenic for man. Is the cause of the bubonic or Oriental plague; bacilli gain entrance by way of the skin, causing localized foci of infection from which buboes develop, followed by pest-sepsis and death. The lungs may be the original site of invasion, and plague pneumonia (worst form of the disease) may result. The typical bacilli can be found in the sputum of the

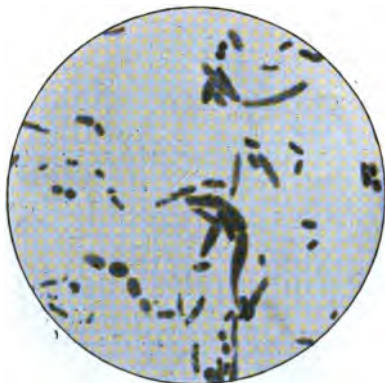


FIG. 25.—Pest bacillus involution forms produced by growing on 3% salt agar. (Kolle and Wassermann.)

patient thus affected. The mortality from this plague is from 50 percent to 80 percent. (Fig. 24.)

Almost all domestic animals—rats, mice, guinea pigs and rabbits are affected; horses and swine are very susceptible; cows and dogs less so. Rats seem to be affected with a chronic form of the malady, and by inhabiting ships and warehouses in foreign countries, spread the disease. Post mortems on infected animals reveal hæmorrhagic petechia and serous infiltration into serous cavities. Death is generally due to a profound toxæmia and exhaustion.

The virulence of the organism can be raised by passing it through a series of animals.

Serum from infected animals agglutinates plague bacilli.

The diagnosis of the plague bacilli is made by rubbing the suspected culture upon the freshly shaven skin of a guinea pig; if the animal develops buboes and dies, and polar staining bacilli are found, it is probable that the organism is the plague bacillus. Further, if curious involution forms develop on heavily salted agar (3 percent) the diagnosis is confirmed. (Fig. 25.)

The disease is spread by flies. Which, according to Yersin, are susceptible to the disease, and spread it by depositing their feces on the human skin, rather than through their bites.

Immunity.—It is possible to immunize against the disease. Kitasato and Yersin produced an anti-toxic serum, which has, not only a prophylactic, but a curative action. By the use of killed culture Haffkine vaccinated many people against the plague.

FRIEDLANDER'S BACILLUS.

Bacterium Pneumoniæ.

Friedlander's Pneumonia Bacillus. (Fig. 26.)

Morphology and Stains.—Short plump rods with rounded ends, surrounded by a thick gelatinous capsule in animal fluids, and when grown in milk; is not motile, and has no spores; does not stain by Gram's method, but easily by the common basic dyes.

Oxygen Requirements.—Grows in and without oxygen, upon all culture media.

Chemical Activities.—Produces abundant acids, CO₂ and H₂ gas, alcohol, indol, and H₂S. also.

Habitat.—Has been found in soil; sometimes in healthy saliva.

Culture Media.—Grows luxuriantly on all culture media.



FIG. 26.—Friedlander's bacillus and pneumococci, showing capsules. (Greene's Medical Diagnosis).

On **gelatine** it grows in roundish elevated colonies that are yellowish-white with a slimy lustre, and never liquefies the gelatine. In **agar** it multiplies even more abundantly with a moister growth. The border of streak cultures is smooth and wavy, and the water of condensation is cloudy. In **bouillon** the growth is very cloudy with a silvery deposit at the bottom. The bouillon becomes thickened. **Milk** is not coagulated, and **potato** yields a luxuriant yellowish, moist shining growth.

Pathogenesis.—It is possible to cause pneumonia in mice, also septicæmia. Guinea pigs and dogs are susceptible. Though frequently found in pneumonia sputum, it is an unimportant pathogenic organism in man. Agglutination takes place with immune serum.

TYPHOID BACILLUS.

Bacterium Typi. Koch and Eberth.

Bacillus Typhosus.

Typhoid Bacillus. (Fig. 27.)

A most important pathogenic organism that causes typhoid fever.

Morphology and Stains.—Generally short plump rods 1 to 3 μ long, and .6 to .8 μ broad. Forms long threads in cultures, especially on potatoes. Contains peculiar shining bodies (polar), and sometimes vacuoles are seen; is flagellated (peritrichous); contains no spores; exhibits pleomorphic and involution forms; is actively motile, and stains with all the basic aniline dyes, but not by Gram's method.

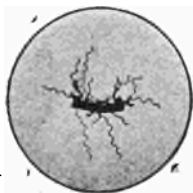


FIG. 27.—Typhoid bacilli showing flagella. (Kolle and Wassermann.)

Vital Resistance.—The thermal death-point is 60° C., ten to fifteen minutes. Remains alive in ice for months; even the temperature of

liquid air does not destroy it. In distilled water it lives for months, but if other saprophytic bacteria are associated with it, however, it

quickly dies. Does not resist drying or chemicals, except carbolic acid, towards which it exhibits a tolerance. Sun-light kills it in an hour.

Habitat.—It never exists in nature, except where water or soil has been contaminated by feces or urine. It may multiply in potable waters, in milk, and the juices of oysters.

Chemical Activities.—Does not produce proteolytic enzymes; forms H_2S . but will not ferment the sugars. Does not yield indol or nitrites. Produces levorotatory lactic acid. Its toxin is all contained within the bacterial cell (endo-toxins) and is not water soluble.



FIG. 28.—Seventy-two hour old culture of typhoid bacillus on gelatine. (Kolle and Wassermann.)

This toxin is manifested by injecting washed and killed bacilli into animals, or by freezing the bacilli with liquid air, and then crushing them. This injected into guinea pigs causes diarrhoea, mydriasis and death.

Oxygen Requirements.—It is a facultative aerobe.

Cultural Characteristics.—It grows upon all media at the temperature of the body, $37^{\circ} C$. and more slowly at $20^{\circ} C$. On **gelatine plate** it produces at first small colonies, yellowish and punctate, which become whitish, delicately notched and ridged. (Fig. 28.) In **gelatine stab**

culture it grows in a thread-like granular line, without producing gas. In neither case is the gelatine liquefied. On **agar plates** the colonies are not so characteristic, being round, grayish-white, and shining. In **milk** it grows well, not coagulating it even after boiling, and only a very little acid is produced. On **potato** the growth is characterized by its invisibility, and this fact is used to differentiate it from other kindred bacteria. The growth is only detected by scratching with a needle. In **bouillon** it grows uniformly, producing very little acid, and no demonstrable amount of gas. In special media (His's semi-solid media) thread-like colonies are produced, which are characteristic. On Elsner's potato media it produces small granular, glistening points. It also grows characteristically in Capaldi and the Drigalski and Cunadi media.

Invasion of Body.—This organism generally invades the body by way of the alimentary tract, in food and water. Flies may infect milk and other foods. Oysters may become infected and cause disease.

Pathogenesis.—It is certainly the cause of typhoid fever. Is found in the stools and urine of the patient, and may be recovered from the blood. Also found in the spleen and gall bladder. It produces well marked histological changes in the lymphoid structures, particularly in Peyer's patches, solitary follicles, and other lymph glands. There is, according to Mallory, a massive endothelial proliferation in the lymph glands. This causes occlusion of the lymph vessels, and is followed by necrosis (ulceration) of the Peyer's patches. The intense phagocytic action of the fixed lymphatic cells in the glands is manifest toward the red blood cells, which are devoured in great numbers. The toxin causes degeneration of other organs, particularly in the liver. Bacilli are found in the spleen and blood in the later stages of the disease. The rose colored spots are found to be full of them. The disease is certainly not a merely localized infection of the lymph structures, but is a bacteræmia. There is often a mixed infection, and the streptococcus pyogenes in the blood plays an active rôle in the necrosis of bone and in

the profound septic condition seen late in the disease. Commonly it produces death by (1) profound toxæmia; (2) ulceration of the Peyer's patches, causing perforation and peritonitis; (3) by the destruction of a blood vessel in the floor of an ulcer producing a hæmorrhage.

In animals, as a rule, typhoid bacilli if injected, produce no disease, and the bacilli rapidly die. In chimpanzees, however, it is possible to produce typical typhoid lesions and symptoms.

Natural and Acquired Immunity.—Human blood serum is strongly bactericidal towards the typhoid bacillus. Normal gastric juice, with its hydrochloric acid, destroys the bacillus when ingested and this forms the natural means of protection. Immunity following an attack of typhoid is generally of long duration. If bacilli do reach the blood stream of an immune individual, the amboceptors originated by a previous infection, together with the complement normally present, effect a solution of the invading organism. Artificial immunity has been effected against typhoid by vaccinating individuals with killed cultures. Anti-toxin for typhoid has been prepared by injecting horses with killed culture of typhoid bacilli, but it has not proved to be effective.



FIG. 29.—Widal reaction. One-half of the field shows typhoid bacilli unclumped, other half shows clumping. (Greene's Medical Diagnosis.)

Agglutinations—One of the most important means of diagnosing typhoid fever is by the so-called Widal test, really the Gruber and Durham agglutination reaction. This consists in applying the serum of the blood of a person, supposedly ill with typhoid, to a fresh bouillon culture of typhoid bacilli. If the person has the disease, and it has lasted for five or more days, the bacilli are promptly agglutinated in clumps. Undiluted normal serum, and serum from people suffering other diseases, will bring about the same reaction at times; it is therefore best to dilute the serum with water 1-50, and if the reaction occurs within an hour the disease is considered typhoid

fever. The test may be made either with a hanging drop and examined microscopically, or macroscopically by adding a drop of diluted serum to fresh bouillon culture of typhoid bacilli, when, if the case is typhoid, large clumps of the bacilli will form and drop to the bottom of the tube. Animals immunized against typhoid exhibit this reaction to a high degree. Serum diluted with 10,000 parts of water has caused the reaction in less than one hour's time. This reaction with a known culture of typhoid bacilli is used clinically to identify serum from a doubtful case of typhoid, and establish a diagnosis. On the other hand, a known serum prepared artificially by immunizing rabbits with bacilli is used to identify typhoid bacilli when found in water, or elsewhere. The fetus of a woman suffering from typhoid contains agglutinins in its blood. The milk, tears, and other body fluids from an individual with typhoid, agglutinate typhoid bacilli. Serum to perform the test may be obtained by puncturing the skin, or by blistering it and drawing off the serum, or else by abstracting blood from a vein with a hypodermic syringe.

The origin of the agglutinin in the body is not yet known. It appears during typhoid, generally after the fifth day, and persists for some time (several years?) after convalescence.

There are two stages to the reaction; immediately after mixing the serum and culture, the bacilli will be seen to become less motile, and then still. After this they begin to huddle together into clumps. In complete reaction they remain immobile and tightly massed. In some cases bacteriolysis occurs, and many of the bacteria are dissolved in the serum. It is still uncertain whether the reaction is merely a phenomenon of infection, or whether it has to do with immunity. By many it is held that the two are distinct and separate and that it is a phenomenon of infection. There are several reasons for thinking so. 1. The bactericidal action of serum is destroyed at 56° C. The agglutinating power is not destroyed at 62° C. 2. A serum may be bactericidal, but not agglutinative. 3. Bacteria treated with bactericidal sera lose their virulence, and those that have been agglutinated do not do so.

Paratyphoid Bacillus.—Also called paracolons, a pathogenic organism producing all the clinical symptoms of typhoid, only in milder form (at times) has lately been discovered. It differs from the true bacillus because it ferments dextrose and maltose producing gas and acid, and is not agglutinated by the serum from a true typhoidal infection, even in high dilution. In every other respect it resembles the typhoid bacillus, and seems to occupy a position between it and the colon bacillus. Paratyphoid resists 60° C. from thirty to sixty minutes, so it is said.

Blood cultures are often employed in large hospitals for the diagnosis of typhoid fever. After the first week bacilli may be recovered from the blood by withdrawing 10 c.c. of blood from a vein and mixing it with 500 c.c. of bouillon. The large amount of blood is necessary, because the bacilli are few in number, and the bactericidal action of the serum outside the body is powerful until mixed with the bouillon, after which the bacilli are able to withstand it.

COLON BACILLUS.

Bacterium Coli.

Bacterium Coli.

Colon Bacillus.

While not strictly a pathogenic organism, it plays such an important part in secondary infection, and resembles so closely the typhoid bacillus, that it will be described here.

Morphology and Stains.—Is not so motile as typhoid; has not so many flagella; and is devoid of spores. It exhibits pleomorphism; may grow in chains; and possesses vacuoles and polar bodies (gonidia) at times. Is readily stained by all the common basic stains, but not by Gram's method.

Oxygen Requirements.—It grows especially well in oxygen, providing sugar is present. Without oxygen its growth is not so good.

Temperature Requirements, and vital resistance. It grows well at room and incubator temperature. Its thermal death-point is

about 62° C.; light and heat are destructive to it, and its resistance to anti-septics is somewhat better than that of typhoid bacillus.

Cultures.—Thrives in all common culture media, especially if sugar is present. If it produces too much acid, it quickly dies. On **gelatine** it grows like the typhoid bacillus (from which it is difficult to differentiate, see page 96) in whitish raised colonies that do not liquefy the media. Sometimes the growth is thin and iridescent, and exhibit bizarre shapes—tadpole-like and lobulated. Typhoid colonies show deep furrow-like ridges under the microscope. In the special

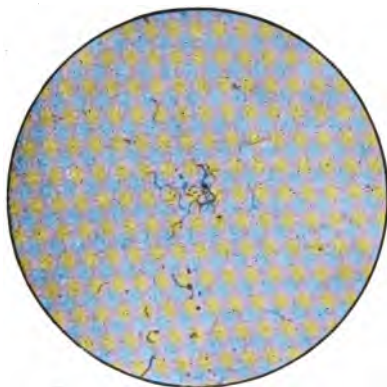


FIG. 30.—Colon bacillus showing flagella. (Kolle and Wassermann.)

semi-solid media of His, the typhoid produces uniform cloudiness, with thread-like colonies. The colon does not so quickly cause this cloudiness, and forms gas bubbles. On **agar plates** surface colonies are like typhoid, only they are thicker and moister. If litmus is added to this media, a red zone forms about the colonies, due to the presence of lactic acid. In **agar tubes** the growth is more luxuriant and resembles typhoid. In **litmus bouillon** it rapidly reddens the litmus, clouds the media, and deposits a slimy sediment. In **milk** it always produces coagulation. On **potato** it grows more rapidly

and luxuriantly than typhoid, at first yellowish-white, which later changes to yellowish-brown. It is slimy.

Chemical Activities.—Produces color on potato only. Sugars are fermented with the production of H_2 , CO_2 , and some N . Occasionally ferments cane sugar. Produces lactic, acetic and formic acids, also indol abundantly, and H_2S . It decomposes urea.

Habitat.—Found always in the intestinal contents of most animals and man. Also in streams and rivers that run through farm lands and by towns. While it is difficult to find typhoid in drinking water, the colon is easily found. If in abundance, it indicates great fecal pollution. In milk it is often found, where it plays an important part in souring.

Pathogenesis.—It is pathogenic to rabbits and guinea pigs, causing peritonitis if injected into the peritoneal cavity. In man it plays rather a subordinate pathogenic rôle, but it has been found the causal agent of some cases of suppurative appendicitis, peritonitis, and cystitis. It may attack the lungs and meninges of feeble children, and cause death by setting up a pneumonia or meningitis. During the agonal period in wasting diseases it may cause terminal infection and death. Colon bacilli encysted in the liver and kidney have been found by Adami in cirrhosis of these organs, and it is believed by him to be partly the cause of these diseases.

Agglutination.—Animals immunized against colon bacilli by repeated injections, exhibit agglutinins in their blood.

The differentiation of the typhoid from the colon bacillus is largely accomplished by noting the chemical reactions of both organisms in culture media. The chief differences are:—

1. The typhoid bacillus has more flagella than the colon, and is much more motile.
2. On gelatine culture plates, the typhoid colonies develop more slowly than the colon, and are much more delicate and transparent. If litmus is present the colon colonies are red, the typhoid bluish.
3. In media containing dextrose, or lactose, gas is produced by the colon, but not by typhoid.

4. In peptone solution the colon produces indol, while the typhoid does not.
5. Milk is coagulated by the colon, but not by the typhoid.
6. On potatoes colon grows much more luxuriantly than typhoid.
7. Typhoid reddens neutral red; colon changes it to bright yellow.
8. The most important test is the agglutinative one. Typhoid is clumped by immune sera, highly diluted, while the colon is not.

DISSENTERY BACILLUS.

Bacterium Dysenteriae.

Dysentery Bacillus of Shiga and Flexner.

Supposed cause of one form of tropical dysentery, Also thought by Duval and Bassett to be the cause of infant diarrhoea in this country. It is not found in all cases of dysentery of bacillary origin. There are various strains of this organism, the differentiation of which depend upon their chemical activities, and agglutinative properties with different sera.

Morphology and Stains.—The organism is, in many respects, similar to the typhoid bacillus, but is plumper. It is flagellated, has no spores, and exhibits pleomorphism. It stains well with the common aniline dyes, but not by Gram's method.

Vital Properties.—It is killed by 1 percent of carbolic solution in thirty minutes. Lives for twelve to seventeen days when dried. Direct sunlight kills it in thirty minutes. Its thermal death-point is 58° C. in thirty minutes. It is a facultative aerobe; grows at ordinary temperature, but better at 37° C.

Cultures.—Grows on all the common culture media, but more slowly than the colon bacilli. **Gelatine** cultures resemble typhoid. The growth in this media (which it does not liquefy) produces no pellicle, but a sediment. Indol is not produced, and **milk** is acidulated, though not coagulated. On **potato** it grows sparingly, often turning it brown. The Shiga type ferments glucose, but no other sugar. The Flexner type ferments glucose, dextrine, and mannite,

but not lactose. The latter type produces more acid than the former, and both are best agglutinated with their corresponding serums.

Habitat.—In living bodies the organism is found solely in mucous discharges from the bowels and uterus. In the dead it is found in the lymph glands. If it reaches the circulation, it appears to be rapidly destroyed by the blood. It has been discovered, however, in the body of a foetus delivered from a woman with the disease. The organism must have passed the placenta of the mother. The disease is spread by water, and it may become epidemic in large institutions.

Pathogenesis.—The typical lesions caused by the organism vary from a mere hyperæmia to a superficial necrosis of the lymphoid structures, which may be extensive. The descending colon and sigmoid are oftenest attacked. The necrotic masses separate, leaving shallow ulcers. The lymph structures are engaged with polynuclear leucocytes. No marked lesion is found in the spleen. The liver and kidneys often undergo marked parenchymatous degeneration. The bacilli being possessed of a powerful endo-toxin, so that dead cultures, if injected under the skin cause marked local and general reactions. Like the pyocyaneus bacillus, this organism undergoes auto-digestion in bouillon, which leaves the latter highly toxic owing to the liberation of the toxins. Laboratory animals quickly succumb to injection of this organism. Dysentery cannot be induced in animals by feeding cultures. Poorly nourished subjects are easily infected and quickly die. Digestive disorders favor infection. Death may be due to toxæmia or exhaustion. As a causal agent in the production of summer diarrhoeas of children, the dysentery bacillus plays a part. It has been isolated from the stools of infants, with this disease, and their sera have been found to agglutinate the bacilli. Nevertheless it is known that other bacteria (streptococci, etc.) cause this disease, and Weaver found that "clinically twenty-four of our ninety-seven cases of iliocolitis in which dysentery bacilli were discovered did not differ from cases in which dysentery bacilli were not found."

Immunity.—The sera from convalescents from dysentery shows a strong bactericidal action. Anti-bodies are developed by infection and artificial inoculation with killed cultures. Kruse obtained a serum from horses which strongly protected a guinea pig against a lethal injection of bacilli: 1–80,000 gram of serum was used for this purpose. The protective property of the serum is due to its bactericidal action. Here the amboceptors act, but only in the presence of a complement. Anti-toxic sera protected against bacteria; and an anti-bacterial serum protected against toxin, according to Rosenthal.

Vaccination.—Shiga tried to induce (1) passive and (2) active immunity in many individuals by injecting both anti-toxic serum and bacteria into them. This was not followed by a lowered number of infections, but by a lowered mortality.

Agglutination.—The serum from a patient suffering from either dysentery or summer diarrhoea, will, after about a week's illness, agglutinate bacilli. This property is not always present, and its absence does not exclude the possibility of infection. In performing the reaction, both Shiga's and Flexner's type of organism should be used. Both these types probably bear the same relation to each other that typhoid and paratyphoid do.

GÄRTNER'S BACILLUS.

Bacterium Enteritidis.

Bacillus of Gärtner.

The cause of one form of meat poisoning, and closely allied to the typhoid bacillus in its morphological characteristics.

Morphology and Stains.—This organism is a short plump ovoid; is motile; has about eight flagella; does not form spores; and stains well with all the common aniline dyes, but not with Gram's method. It forms a slight capsule.

Vital Resistance.—It is a facultative anaerobe. Light and drying kill it. So does heat, but not easily.

Cultures.—Grows on all the common culture media. In **bouillon** thrives well, producing gas in media containing sugar. It ferments glucose, dextrose, lactose, galactose, maltose, and cane sugar. Does not produce indol, which distinguishes it from the colon bacillus, to which it is closely allied. In **milk** it reduces litmus and coagulates the casein in a few days. On **potato** it grows well, producing a yellowish shining layer. On **gelatine** it multiplies without liquefying the media. Superficial colonies in plate are pale and gray, deep colonies yellow and spherical.

Chemical Activities.—Acid, gas and a powerful heat-resisting toxin which is soluble, are found. Infected meat contains this toxin, which is not destroyed by cooking.

Pathogenesis.—It is pathogenic for man, horses, cattle, and laboratory animals. Neither the bacilli or the toxin they elaborate are destroyed by heat. Flesh is infected before death, after which, both the bacilli and toxin increase. Mischief follows the partaking (usually in the form of sausages, etc.) of this meat, causing, in men, violent nausea and diarrhoea, skin eruption, and in severe cases, pneumonia, nephritis, collapse and death. Mortality is from 2 percent to 5 percent. The post mortem findings are not specific. There may be evidence of an enteritis with swollen lymph follicles, and an enlarged spleen.

Agglutination.—The blood of infected individuals after eight days may agglutinate bacilli. A dilution of such blood with 8,000 parts of water has produced the reaction.

PYOCYANEUS BACILLUS.

Bacterium Pyocyaneus.

Bacillus Pyocyaneus. (Fig. 31.)

Bacillus of Blue Pus.

An organism of minor importance as a pathogenic agent, that is often met with.

Morphology and Stains.—Slender rods, often growing into thread-like forms. Exhibits pleomorphism. Sometimes is rounded and cocci-like, is motile, has a polar flagellum, and no spores. Stains with all the basic aniline dyes, but not with Gram's method.

Oxygen Requirements.—Usually a strict aerobe.

Cultures.—Grows on all the **common culture media** luxuriantly, at room and incubator temperatures. It elaborates two pigments, a water-soluble greenish bacteriofluorescein, and a chloroform-solu-

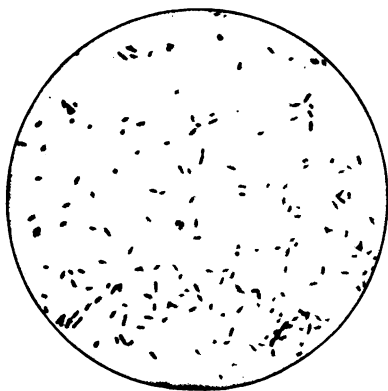


FIG. 31.—*Bacillus pyocyaneus*. (Kolle and Wassermann.)

ble pigment, beautiful blue in color, called pyocyanin. On **gelatine plates** it produces yellowish-white to greenish-yellow colonies which liquefy the **gelatine**, causing crater-like excavations about the colonies. **Gelatine stab** cultures rapidly liquefy along the line of inoculation, coloring the gelatine greenish-blue, and a white crumbly deposit forms in the bottom of the stab. On **agar plates** it produces yellowish-white colonies, surrounded by a zone of bluish-green fluorescence. It grows luxuriantly. In **agar tubes** it multiplies rapidly, spreading over the media, with wavy thickened edges.

The **agar** quickly turns a dark greenish-blue, and in old cultures the growth changes from yellow to greenish-blue.

In **bouillon** it is very dense and yellowish-green; a pellicle forms on the surface, and a sediment is deposited. In old bouillon cultures the bacilli undergo autolysis and disappear. In **milk** the growth is luxuriant, the casein is coagulated, and the clot is ultimately digested. The reaction is alkaline. On **potato** it varies in luxuriance, often being slightly elevated, yellowish, turning to green. The variance in growth is due to the kind of potato used. Drying kills the organism speedily; four hours in sunlight also destroys it.

Chemical Activities.—No gas is generated. Besides the pigments (already specified) ammonia is produced, also a peculiar enzyme called pyocyanase by Emmerich and Löwe, which not only digests gelatine and milk-curd, but the bacterial cells themselves. Old cultures are poisonous; a hæmolysin is produced—an endotoxin, and a soluble toxin. Against the endo-toxin no anti-toxin can be obtained, but against the soluble toxin it is possible to prepare one. The last named toxin stands a temperature of 100° C.

Pathogenesis.—Has been found a sole cause of meningitis and vegetative endocarditis in man; is a pyogenic organism; can cause suppuration anywhere in the body; produces blue pus; is pathogenic to guinea pigs; and its virulence can be raised by passing it through a series of animals.

Agglutination.—The serum of infected and immunized animals both in moderate dilution causes agglutination of bacilli.

BACILLUS OF SOFT CHANCRE.

Bacterium Ulceris Chancrosi. (Ducrey.)

Streptibacillus of Soft Chancre.

Morphology and Stains.—A small thin bacterium .5 μ broad, 1.5 μ long, growing in chains with polar staining, which can be demonstrated in sections of chancres without much difficulty.

This organism does not stain by Gram's method, but by Löffler's it is stained with ease.

Cultures are hard to make. It grows best in **serum agar**, and **blood agar** in faint colonies that are not very characteristic. In condensation water of agar it grows feebly.

In sections and in pus the organism is frequently found in the interior of leucocytes.

By aspirating pus from buboes and planting it in blood agar cultures may be obtained.

Pathogenesis.—From an old culture of over ten generations typical ulcerations were produced in man. The organism is feeble and quickly dies in culture media or in contact with mild antiseptics.

'ANTHRAX BACILLUS.

Bacillus Anthracis.

Anthrax Bacillus of Koch. (Fig. 32.)

Practically the first pathogenic organism to be isolated. This was accomplished by Dr. Robert Koch. It is the cause of a widespread malignant disease, variously called Anthrax, Charbon, or Splenic Fever. Animals and man are infected by it, and its action is often rapidly fatal.



FIG. 32.—Anthrax bacilli in blood. (Greene's Medical Diagnosis.)

Morphology and Stains.—In animal tissues this organism appears as a large rod 3–10 μ long, and 1–1.2 μ wide. Is often in pairs or chains. In fresh specimens the ends of the rods are rounded; when older, the ends become square or concave. Often they have a faint capsule surrounding them. In culture media they exhibit spores and grow in long threads, these threads form long spirally twisted masses, like locks of wavy hair. No flagella are formed, and the organism is not motile. In old cultures, bizarre involution

forms are found. It stains well with all the common basic dyes and by Gram's method.

Oxygen Requirements.—Is a facultative anaerobe, but grows much better in the presence of oxygen. If oxygen is excluded, no liquefaction occurs.

Temperature.—Grows between 14° C. and 45° C.; best at 37° C. Spores are formed, if oxygen is abundant, above 12° C. Sporulation is more rapid at 37° C. Spores withstand high temperature (dry) for a long time, 100° C. for one hour. The bacillus itself is killed



FIG. 33.—Anthrax bacilli growing in a chain and exhibiting spores.
(Kolle and Wassermann.)

at 70° C., moist heat, in one minute. The thermal death-point may be put down for the organism, at 100° C. moist, for five minutes.

Vital Resistance.—Highly resistant to chemicals, light and drying. Spores resist 5 percent carbolic solution for days (Esmarch), and 1 percent corrosive sublimate (aqueous) for three days. They also resist formaldehyde and sulphur for a long time, and withstand light. Three and one half hours' exposure to bright sunlight killed the spores if oxygen was not excluded. (Dieudonné.) (Fig. 33.)

Sporulation Phenomena.— At 12° C. spores are formed if oxygen is present. The most favorable temperature for sporulation is that of the body (37° C.). Spores are never found in the bodies of living or dead animals if they remain unopened, and oxygen is excluded. If bacilli are cultivated at 42° C. for a long time and frequently reinoculated, on fresh media, the ability to form spores is lost even if grown again at 30° C. (Phisalix). If cultivated upon media containing carbolic acid and hydrochloric acid, the ability to sporulate may be lost.

Chemical Activities.—Acetic acid is formed, as is H₂S. Liquefying, milk coagulating, and milk digesting enzymes are formed. Toxins have not been isolated, but may be formed?

Habitat.—Only found where infected animals, hides, and hair have been. Fields, hay, bristles, hides, manure, etc., have been found to contain bacilli. Drinking water may be polluted by tanneries and the bodies of dead animals. Meadows and fields may be contaminated for years. From the buried bodies of infected animals anthrax spores may be brought to the top of the soil by earth-worms.

Cultures.—Grows exceedingly well on all culture media in the air. On **gelatine** it grows in whitish round colonies, rapidly sinking into the gelatine, due to the liquefaction. The liquid media is turbid. The interior of the colony is crumbly. When magnified, the colonies seem to be made up of tangled waving bundles, like locks of hair, especially about the periphery. In **gelatine stab cultures** the growth is luxuriant and rapid; the media is liquefied more rapidly at the top, and finally a crater is formed; before this appears, lateral hair-like outgrowths are seen in the gelatine. At the bottom of the crater a white crumbly mass is formed, but no pellicle. On **agar plates**, small whitish colonies develop which are elevated and round. When magnified, wavy hair-like growths appear on the edge, caused by many twisted parallel chains of bacilli. (Fig. 34.)

In **agar stab**, the growth is more luxuriant near the top; lateral filamentous branches are seen along the stab line. In **agar streak**

the colonies are abundant, thick and fatty; have tangled edges, and the water of condensation is cloudy. In **bouillon**, it forms homogeneous flocculi, which precipitate, leaving the bouillon clear. No pellicle is formed. In **milk**, it multiplies rapidly, the proteids are coagulated, generally rendered acid, and later the coagulum is dissolved. **Potato** cultures are likewise luxuriant. The growth is elevated, dull in lustre, and the outline is wavy.

Pathogenesis.—The anthrax bacillus increases so rapidly, and so luxuriantly, that it has been supposed to cause death merely by



FIG. 34.—Anthrax bacilli. Cover-glass has been pressed on a colony and then fixed and stained. (Kolle and Wassermann.)

mechanically overwhelming the animal: absorbing nutriment and oxygen, and blocking capillaries. Its action is certainly not purely toxic, as it causes, not a toxæmia, but a bacteræmia. It is especially virulent for man, sheep, cattle, goats, rabbits, guinea pigs, mules, and horses. Rats rarely succumb. Pigeons, chickens, and dogs are immune. If frogs are kept at a temperature of 30° C. they become susceptible to infection. At their normal temperature they are immune. The disease produced by this organism is known

variously in different countries as Anthrax, Splenic fever, Wool-sorter's disease, Malignant pustule, and Charbon. It frequently devastates vast herds of sheep, cattle, and goats, and is often a pestilence in European countries, China, and South America. It appears sporadically in the United States. Its origin in this country can usually be traced to infection from hides or hair imported from abroad. In man it is frequently fatal. The infection is first manifest as a small carbuncle or pustule, from this, rapid general infection, as a rule, ensues. In man and animals anthrax bacilli may be transmitted from mother to foetus via the placenta. The organism is found in enormous numbers in infected bodies, investing all the organs and the blood. Pus is produced, and tissues are degenerated. Infection is accompanied by a high leucocytosis and fever. There is often congestion of the lungs; also an intense friability of the splenic pulp, and all the glands of the body become enlarged, and, at times, many of them suppurate. In wool-sorter's disease, the bacilli are inhaled, and lung lesions result.

Immunity.—It is possible to immunize animals against infection with anthrax by means of vaccines. By this means the lives of many thousands of domestic animals have been saved.

TETANUS BACILLUS.

Bacillus Tetani.

Tetanus Bacillus. (Fig. 35.)

Lockjaw Bacillus.

First seen by Nicolaier, and isolated in pure culture by Kitasato.

Morphology and Stains.—Rod-shaped. Varying from 1.2μ in length, to very long threads of 20 to 40μ . Sometimes grow in chains; frequently appear like short drum-sticks with a spore at one end, which is either round or oval. At times, the bacilli in chains sporulate. The organism is motile; possesses numerous flagella (from fifty to a hundred) peritrichously arranged; stains well with

all the common basic aniline dyes, and retains the color in Gram's method. (Fig. 36.)

Oxygen Requirements.—Strictly anaerobic when freshly isolated from earth or wounds, but, after long cultivation on culture media, it becomes more tolerant to small amounts of oxygen.

Temperature.—Grows best at 37° C. Below 14° C. not at all.

Vital Resistance.—Spores resist 80° C. for an hour. This fact enabled Kitasato to kill all other organisms, except their spores, in



FIG. 35.—Tetanus bacilli showing end spores. (Kolle and Wassermann.)

pus. Six days' exposure to direct sunlight is needed to kill the spores, the thermal death-point being 100° C. for five minutes.

Chemical Activities.—Ferments sugar; produces gas, indol, alkali, and H₂ S. which gives to the culture an odor of burnt garlic or onion. Gelatine is liquefied; marsh gas, CO₂, and nitrogen are produced. The most important product of growth is the highly poisonous complex toxin, which is made up of tetanolyisin, and tetanospasmin, the latter has a great affinity for nerve tissues. This toxin is soluble in water, and can be separated from it by means of ammonia sulphate.

Habitat.—Is found in garden soil, hay, manure, and dust. Has been found in cobwebs, on weapons, in cartridges, and in the feces of man and of animals. It is said to have been found in the spinal cord of a man who did not die of tetanus. It has also been isolated from bronchi in a case of rheumatic tetanus in which there was no lesion in the body (Carbon and Perrors). In **disease** it is found in the infected wound, generally in a deeply punctured one, which is usually purulent and contains but few bacilli. Puer-

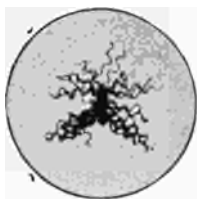


FIG. 36.—Tetanus bacilli showing peritrichous flagella. (Kolle and Wassermann.)

peral tetanus, and tetanus of the new-born, are but varieties of the disease, dependent upon the site of infection, whether of the placenta or umbilical cord. Tetanus sometimes occurs spontaneously, without a sign of lesion anywhere. Sheep and goats are susceptible to infection, so are guinea pigs and rabbits. Horses are peculiarly susceptible. Soil, or manure, getting into wounds, is often a cause of tetanus. Cow-dung poultices, mud dressings, or cobweb applications to stop hæmorrhages, have also caused the disease, Tetanus following vaccination is often due to infected virus, the latter becoming infected from the feces of the vaccine-producing cows.

Cultures.—This organism is difficult to grow, and always requires an atmosphere of hydrogen.

On **gelatine plates**, the colonies appear first as minute white specks, which slowly liquefy the media. As it grows, hair-like threads branch out into the media, and the colony resembles the periphery of a chestnut burr; later, the white appearance changes to yellow. In **gelatine stab** the growth is, at first, whitish along the line of the needle, eventually the gelatine becomes liquid, and a bubble of gas, partly filled with whitish-cloudy liquid gelatine, appears. On **agar plates** the colonies are ragged, and are surrounded by delicate out-spreading filaments. In deep stab culture, down in the agar and remote from the top, a spreading tree-like form appears,

with spike-like growths in the agar. **Blood serum** is sometimes liquefied. **Bouillon** is uniformly clouded, gas is generated if sugar is present, and toxin is produced. **Milk** is, generally, not coagulated.

All cultures of tetanus must be grown under an atmosphere of hydrogen in media, from which all free oxygen has been driven by boiling, or else abstracted by a mixture of pyrogallic acid and sodium hydrate. It is possible to cultivate the organism under mica covering, or paraffine poured upon freshly boiled media. If sterile glass tubing is *filled* with agar or gelatine, and inoculated with tetanus bacilli, then sealed, colonies will develop, as perfect anaerobic conditions are thus obtained. Often the organism grows best in the presence of saprophytic ones. Strongly pathogenic organisms do not grow well in culture media, while comparatively non-virulent ones grow very well.

Pathogenesis.—Tetanus may follow any wound, no matter how insignificant, though deeply punctured ones, caused by nails or splinters, are more often followed by tetanus infection, especially if the puncture is sealed by blood clots or pus, and so creating an anaerobic condition necessary for growth. If the wound is on the face or hand, tetanus symptoms more quickly supervene, while if the wound is on the foot, these are apt to be delayed. The sooner the symptoms appear after the reception of the injury, the more likely will the disease be virulent and fatal. If spores are washed free from toxin, according to Viallard and Rouget, and then injected into a susceptible animal, they do not cause tetanus, but are taken up by the phagocytes. Necrotic tissue in wounds favors infection with tetanus, since it helps to fulfil anaerobic conditions, and in some way hinders phagocytosis. Aerobic bacteria favors tetanus infection by absorbing the free oxygen which prevents the growth of tetanus organism. Free oxygen never kills the organism or its spores, but merely prevents its development. Wounds that have, apparently, healed, may be the cause of tetanus. The toxin is produced rapidly in wounds, or what is more likely, some is introduced with the bacilli and other dirt. Kitasato found, in the case of mice,

that if bacilli are introduced in the skin, near the tail, and in an hour the whole area is excised, and the wound cauterized, fatal tetanus nevertheless supervened.

Rheumatic tetanus follows pulmonary infection. As related in the chapter on toxins, the mode of disease production is as follows:—The toxin is conveyed from the wound by means of the motor nerves to the central nervous system affecting the motor elements. It causes microscopic degeneration of the fibers and cells of the motor apparatus. Death is caused either by a spasm of the glottis or diaphragm, or by cardiac failure and exhaustion. A local manifestation merely affecting certain groups of muscles may occur. Laking of the blood by tetanolsin found in the bodies dead from tetanus is a well known phenomenon. In fatal cases, toxin may be demonstrated in the bladder by injecting the serum into mice, causing in them tetanic symptoms. Various groups of muscles are affected in tetanic seizures. The muscles of the jaw, if affected, cause *trismus*; if those of the back are involved the individual suffers from *opisthotonos*. The seizures may be constant or *tonic*; or convulsive and violent, then they are designated as *clonic*.

Immunity.—Metchnikoff claims that the only natural immunity possessed by man against tetanus resides in his leucocytic powers of defense. Susceptibility of the natural receptors of the nerve cells for the toxin, and the degree of affinity, constitutes the cause of intoxication, its degree, and ultimate result. Affinity for the receptors of other less vital organs, on the part of the toxin, establishes a means of natural defense. Acquired immunity is dependent upon the formation of anti-toxin. The anti-toxin, formed by susceptible animals injected with tetanus toxin, is chiefly useful and valuable as a prophylactic measure. An epidemic of puerperal tetanus in a lying-in hospital was checked by its use. Sprinkling dry powdered anti-toxic serum on wounds infected with tetanus bacilli, or toxin, prevented infection (Calmette and McFarland). The anti-toxin may be injected either into the substance of the brain in cases of well developed tetanus, or into the cerebro-spinal fluid, in the

hope of neutralizing the toxin not already in firm combination with the nervous elements. Large nerves near the infecting wound may be injected with anti-toxin in the hope of binding the toxin already in combination with the nerve cells.

Female mice immunized against tetanus toxin, transmit a great amount of immunity to its off-spring. Her milk also causes a passive immunity in other young that are suckled by her.

BACILLUS OF MALIGNANT ŒDEMA.

Bacillus Œdematis Maligni.

Vibrio Septique.

Bacillus of Malignant Œdema.

Morphology and Stains.—Thickish rods, resembling tetanus and symptomatic anthrax bacilli, with a tendency to grow in long threads. It is actively motile, and is possessed of numerous peritrichous flagella. Spores are found which may be either equatorially or polarly situated. This organism is readily stained by the ordinary methods, but not by Gram's.

Chemical Activities.—Milk is coagulated, but not soured, and the reaction is amphoteric. Abundant alkali is formed at times; albumin is decomposed, forming fatty acids, leucin, an oil, and an offensive odor. CO₂, N. and marsh gas, are also formed.

Habitat.—It is found in soil, dust, manure and dirty water and is widely distributed.

Cultures.—This organism is a strict anaerobic, and grows well in most culture media, at incubator or room temperature. On **gelatine plates** colonies develop on the surface (under hydrogen) in tiny shining white bodies, which upon magnification are found to be filled with a grayish-white substance composed of melted gelatine, and long tangled filaments. The edges of the colonies are fringed. In **gelatine stab cultures** (made in liquid gelatine, which, after inoculation, is rapidly solidified in ice water) a globular area

of liquefaction occurred. If sugar is added, active fermentation takes place, with the production of large amounts of offensive gas. It grows well on **agar**, in **bouillon**, and in **milk**.

Pathogenesis.—Is pathogenic for man, horses, sheep, dogs, rabbits, calves, pigs, goats, rats, mice, and guinea pigs. Cattle are said to be immune. When bacilli are applied to a scratched surface, infection is not likely to occur, as free oxygen seems to inhibit the growth; if, however, the wound is deep, rapid infection follows, young domestic, and laboratory animals dying within forty-eight hours. In man, the clinical manifestation of infection with this organism is known as malignant oedema. Infection has followed penetrating wounds of the body, by dirty tools, nails, splinters, bullets, etc. The disease is often quickly fatal. It produces, frequently, rapid moist gangrene.

Bacilli have been found in the blood of dead animals. Infection is very apt to follow contused wounds, especially if other bacteria, like the *Bacteria Vulgæ*, or *Bact. Prodigiosus*, are present. A mixed culture in vitro of this organism, and the *bacillus acidi paralactici* produces butyl alcohol abundantly. Neither of these organisms separately can do so.

SYMPTOMATIC ANTHRAX BACILLUS.

Bacillus Chauvoei.

Bacillus of Symptomatic Anthrax.

Rauschbrand Bacillus. (Figs. 37 and 38.)

The cause of symptomatic anthrax, black-leg, or quarter-evil, in cattle.

Morphology and Stains.—This is a large organism, 5μ in width, and 3 to 5μ in length. It has rounded ends, and grows in pairs, but not in strings or chains. It is motile, and has many peritrichous flagella. When stained for spores, these bodies may be found distending the organism in the middle or at the end, and the bacillus

assumes a drum-stick, or spindle shape. Often chromophilic granules are present; involution forms also appear, and are of enormous size. This organism stains with all the common stains, but not by Gram's method. They may be seen in an unstained condition in blood or other fluids.

Habitat.—This bacillus is found not only in the diseased tissues and dead bodies of infected animals, but also in infected pastures, soil, hay, etc.

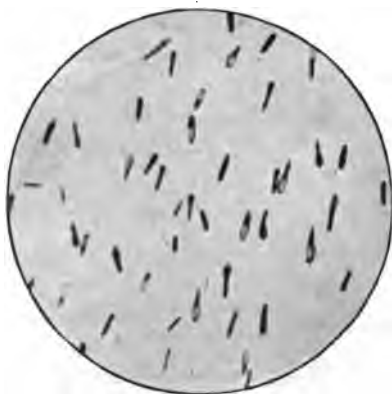


FIG. 37.—Rauschbrand bacilla showing spores. (Kolle and Wassermann.)

Temperature Requirements.—It is best cultivated at body temperature, but grows anywhere between 18° C. and 37° C.

Cultures.—It is, like tetanus and malignant oedema organisms, a strict anaerobe. On **gelatine** it grows in roundish whitish colonies in a delicate tangled mass, with projecting filaments. The gelatine is liquefied, and bubbles of gas are formed in stab cultures. A sour odor is emitted from cultures; 1 percent to 2 percent of sugar is required for successful cultivation; or 5 percent of glycerine will answer. On **agar** the growth is marked; gas is produced, and acidous odors evolved. In **bouillon** it grows rapidly. Large

masses of the organism sink to the bottom, gas is formed, and the media is clouded. **Milk** affords a good media for the growth of the organism, but the casein is not coagulated.

Pathogenesis.—Young cattle, six months to four years old, sheep, goats, rats, mice, and more especially guinea pigs, are susceptible to it. Swine are immune, while dogs, cats, birds, and rabbits are not so susceptible. Man is immune. It causes in animals peculiar groups of emphysematous crepitating pustules, followed by emaciation and death. These areas contain dark fluid, probably

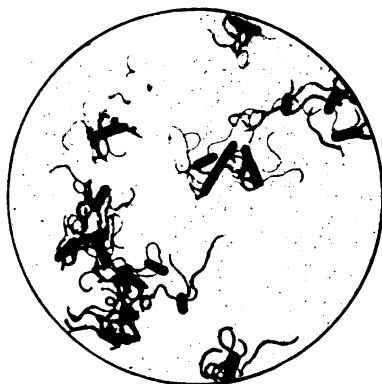


FIG. 38.—Rauschbrand bacillus showing flagella. (Kolle and Wassermann.)

broken-down blood. In guinea pigs inoculation is followed by death within thirty-six hours. The site of inoculation is found to be oedematous, and contains bloody fluid. The organs generally are normal. The bacilli are mostly found at the site of the inoculation, but later in the blood in every part of the body. The virulence of this organism in culture media is soon lost. The addition of lactic acid to the cultures increases their virulence.

Immunity.—It is possible to decrease the virulence of this organism, and to use the weakened bacteria as a vaccine against infection.

To attenuate this bacillus, prolonged exposure to heat, or to heat and drying together is necessary. Inoculation with bacilli treated in this way is followed by a mild local reaction, which affords complete immunity against infection with virulent bacilli. It has been found by Kitt that the muscles of an infected animal, if subjected to a high temperature—85° C. to 90° C.—afforded complete protection to the animal inoculated with it. It is best to use a weaker vaccine muscle that has first been heated to 100° C. for two hours, in order to protect against the active vaccine. Before heating, the meat is ground. When used as an injection, it is crushed and mixed in a mortar with sterile water. Guillod and Simon found that this means of preventative inoculation reduced the death rate in unprotected animals from 5-20 percent to 5 percent. If this bacillus, and the prodigiousus bacillus is injected into naturally immune animals, death will often result.

MEAT POISONING BACILLUS.

Bacillus Botulinus. *Van Ermengem.*

Bacillus of Meat Poisoning, or Botulism. (Fig. 39.)

Morphology and Stains.—This bacillus resembles thick vigorous rods, 4-9 μ long, and 9 μ thick, is motile, has polar spores, and from four to nine peritrichous flagella. It is strangely called a saprophyte, because it is incapable of growth in the body, yet its toxin is highly poisonous to man and other animals. It is stained by all the usual basic aniline dyes, but not by Gram's method.

Habitat.—Is found in raw meat, improperly cured hams, and in sausages. It gains access to meat after the death of the animal.

Vital Characteristics.—Is an anaerobe. Its thermal death-point, for a spore-bearing organism, is low, 80° C., for half an hour. Grows only in media that is alkaline, and is capable of growth at from 18° C. to 35° C., though best below 35° C.; 6 percent of chloride of soda checks growth.

Chemical Activities.—Can produce toxin (which is soluble in water) at a relatively low temperature. Milk is not coagulated, grape sugar is fermented, and a foul odor is produced in a culture that is sour. It liquefies gelatine.

Cultures.—On **gelatine plate**, that contains sugar, colonies are produced that are coarse and prickly in appearance. The liquefaction of the gelatine is slow. **Bouillon** is rendered turbid. The cultures resemble tetanus and malignant oedema.

Pathogenesis.—Its pathogenic action is marked, but only by its



FIG. 39.—Bacillus of botulism. (Kolle and Wassermann.)

toxin, which has a decided affinity for nervous tissue. The toxin is absorbed from the intestinal tract unchanged by the gastric juice. In this it differs from the toxin of diphtheria and tetanus. If the toxin is mixed with the emulsified nerve tissue, it becomes neutralized. In fatal cases of infection, the ganglionic nerve cells are degenerated. Man is very susceptible, while cats and dogs are more or less non-susceptible. If bacilli are inoculated into animals, they do not proliferate. Animals that recover are found to have developed strong anti-toxin in the blood serum.

Immunity.—An artificially prepared anti-toxin has been found to be active, and is of use in treating cases of poisoning with meat.

GASEOUS EDEMA BACILLUS.

Bacillus Capsulatis Aerogenes.—Welch.

Morphology and Stains.—A vigorous plump bacilli 3 to 4 μ in length, resembling the anthrax bacillus, and is usually straight.

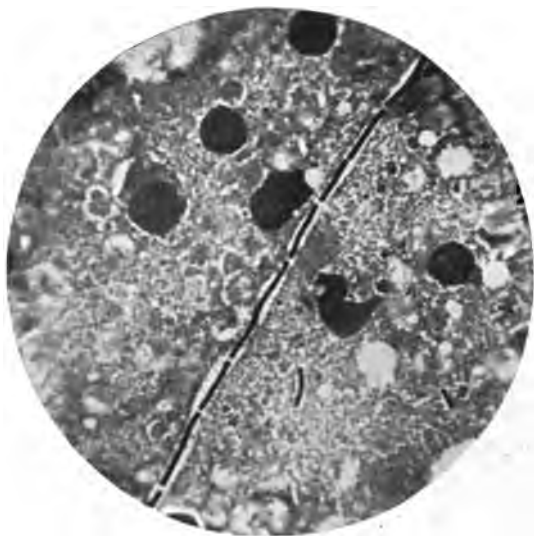


FIG. 40.—*B. Aerogenes capsulatus* of Welch, in smear. (Williams.)

It forms spores, is non-motile, and flagella have not been found. It occurs in pairs, but not in chains. In old cultures involution forms are seen. Spores are generally equatorially situated. Is colored by all the basic dyes, and holds the stain in Gram's method. Staining shows that it possesses a capsule.



FIG. 41.—*B. Aerogenes capsulatis*, agar culture showing gas formation. (Williams.)

Habitat.—The soil, the intestines, and, sometimes, the skin of man.

Vital Characteristics.—Vital resistance is low, the thermal death-point being 58° C. with ten minutes' exposure. It grows best at body temperature. Has lived for one hundred days on culture media in the incubator. It is an anaerobe.

Chemical Activities.—Produces gas; does not usually liquefy gelatine, but curdles milk. (Fig. 41.)

Cultures.—Grows best in neutral or alkaline, producing abundant gas. Colonies appear grayish or brownish-white, and are often surrounded by projections which are feathery or hair-like. On **agar** strict anaerobic conditions are necessary for growth, gas bubbles appear in the media, and the agar may be forced out of the tube in stab cultures. In **bouillon** it grows under anaerobic conditions. The growth is rapid, bouillon is clouded, and a froth appears on the surface. After a few days the media becomes clear, owing to the sedimentation of the bacilli. Growth occurs best in sugar bouillon, which becomes strongly acid. In **milk** the growth is rapid and luxuriant; the proteids are coagulated. Anaerobic conditions must be observed. On **potato** it grows well, producing

bubbles in the water which may cover the potato in the tube. The growth appears thin, moist, and grayish-white.

Pathogenesis.—The pathogenic properties of this organism are limited. It is not able to endure the oxygen of the circulating blood. Grows best in old clots, and in the uterus. It produces gas rapidly in some cases of abortion and in peritonitis in men, which is quickly followed by death. It causes gaseous phlegmons in guineas pigs, and injections are usually fatal to birds. In man infection has followed wounds, and delivery of the child in puerperal cases. It produces in fatal cases the condition known as frothy organs—"Schaumorgane."

SPIRILLACEÆ.

CHOLERA BACILLUS.

Vibrio Cholerae. Koch.

Spirillum Cholerae. (Fig. 42.)

Cholera Bacillus.

Comma Bacillus.

Morphology and Stains.—Curved or bent rods, the ends not lying in the same plane. This bending varies greatly. Under certain conditions of growth such as the presence of alcohol, or insufficient albumin or oxygen in culture media, long spiral chains are formed. It is motile, has one or two terminal flagella, and like other members of this family, has no spores. It stains well with the common dyes but not by Gram's method. Dilute fuchsin stains it best. Occasionally involution forms are developed, which do not stain well. So-called arthrospores are formed, according to Hüppe.

Habitat.—It is said to exist constantly in the waters of the Ganges in India. Is frequently found in contaminated drinking water, from rivers, lakes, and wells; also in human feces, which, used as

manure, infests vegetables, and spreads the disease. It is found in the intestines during cholera, and after death in other viscera.

Vital Resistance.—Is extremely sensitive to various deleterious agencies. Minute quantities of mineral acids, and other chemical disinfectants, as well as light, heat, and drying, quickly kill it; one percent carbolic kills rapidly. A 1-2,000,000 solution of corrosive sublimate destroys in from five to ten minutes. Its thermal death-point is 60° C. for ten minutes (moist heat).

Chemical Activities.—It creates indol in large quantities, and may



FIG. 42.—Cholera spirilla. (Kolle and Wassermann.)

be detected in peptone cultures merely by the addition of sulphuric acid. Dextrorotatory lactic acid is produced from all the sugars. Gases are not formed. Yields alkali in culture; causes slight coloration of potato, and produces a disagreeable odor in bouillon; also yields H_2S , and ferments that liquefy gelatine. Bacteriolysins and invertin are also produced, as well as a toxin which is soluble in water. The most powerful toxin, by far, is contained in the cells of the vibrio themselves. This causes death after intra-peritoneal injection in guinea pigs.

Oxygen Requirements.—It is a facultative aerobe; its growth, however, without oxygen is slow, while powerful toxins are formed.

Temperature.—Grows best at 37° C., but very well at 23° C. Does not grow below 8° C.

Cultures.—On **gelatine plates** the growth is characteristic. Small yellowish-white colonies, which rapidly liquefy the gelatine, appear in twenty-four hours. As the colony increases in size it becomes more and more granular, and finally the whole media is liquefied. In **gelatine tube** stab culture, the growth, at first, is not characteristic; but, after a few hours, a semi-spherical depression appears, which extends downwards, and resembles a large bubble of gas. As liquefaction progresses, the whole line of puncture disappears, and the excavation looks cylindrical. This area becomes cloudy. On **agar plates** the colonies are elevated, round and white, with a moist lustre. Deep colonies are whetstone shape. Old agar colonies become yellowish-brown. **Coagulated blood serum** is rapidly liquefied at 37° C. **Milk**, at times, is coagulated. No curdling ferment is formed; the acid produced is thought to be sufficient. On **potato** the growth is slow, or not at all, if the media is acid. If the potato is rendered alkaline, growth occurs, with a moist lustre, slightly elevated; white at first, later becoming brown. On **acid fruits** it will not grow. In **bouillon**, after sixteen hours, a diffuse cloudiness occurs, with the formation of a stiff pellicle, which in some cultures becomes wrinkled. In **peptone**, abundant growth takes place, with the production of indol and nitrites. If a few drops of H_2SO_4 are added, a beautiful red appears if nitrites are present. This is the "cholera red" reaction. If the color does not at once appear, nitrites must be added.

Pathogenesis.—Cholera spirilla are pathogenic for man and guinea pigs. If the stomach of the latter is rendered alkaline with bicarbonate of soda, and a bouillon culture introduced, choleraic symptoms will follow and the animal will die. If cholera spirilla are injected into the peritoneum, the animal will quickly succumb to a general cholera peritonitis. Young rabbits are equally susceptible. When

cholera spirilla in culture have been swallowed by man (laboratory workers), either by design or accident, the disease has followed, sometimes with fatal results. The toxin of this organism is intra-cellular (an endo-toxin). Old cultures become pathogenic through a bacteriolytic action, by which the cells are dissolved, and the toxin liberated. Filtrates from young culture are non-toxic. If bouillon cultures are killed by chloroform, and then injected into animals, toxic action follows. In cholera the pathogenic process is mostly confined to the intestines. Toxic absorptions, due to the liberation of toxic products by the bacteriolytic action of serum, follow later. There is a desquamation of the epithelium of the bowel, and epithelial cells found in the watery discharges resemble rice grains. Peyer's patches may become slightly swollen and reddened, and later, there may be a diphtheritic necrosis above the ileocecal valve, and often a parenchymatous nephritis.

Diagnosis.—Bacteriological diagnosis of cholera is accomplished by examining the alvine discharges. A mucous flake is mixed with some peptone solution, this is incubated, and the spirilla, if present, rapidly grow on the surface; after a few hours, plates are poured from this surface growth, and from the plates liquefying colonies are picked out, and bouillon cultures made. These are tested by dried serum, from horses artificially immunized by injecting cholera spirilla into them. If the organism under examination (after serum mixed with 2,000 to 3,000 parts of water is added) agglutinates, it is considered to be the cholera spirillum. Both in early and fatal cases, the agglutinating reaction is not available, since it takes some time for the agglutinins to form in the blood. Under the chapter on immunity an account of the Pfeiffer reaction is given, also one on vaccination against cholera infection, by means of killed cultures, under the chapter on vaccines.

Allied Vibrios to the Cholera Vibrio.

Several other vibrios have been discovered that resemble the cholera vibrio. These are mostly found in potable waters, and

though in many respects identical with the cholera vibrio, they differ in essential points, i.e., pathogenicity, and in their agglutina-bility with specific sera. The most important of these organisms are:—*Vibrio Metchnikovii*; *Vibrio Proteus*; *Vibrio Tyrogenes*; and *Vibrio Schuylkilliensis*; *Spiriellen Deneke*. There are no important pathogenic members of this group.

GLANDERS BACILLUS.

Bacterium Mallei.

Bacillus Mallei.

Glanders Bacillus.

Morphology and Stains.—Slender rods 2 to 3 μ in length, containing no true spores, but shining chromatophilic bodies (*Babes-Ernst* granules). In old culture, long club-like threads appear, which exhibit true branching. This organism is not motile, and has no flagella. It is stained with difficulty by ordinary methods, and not at all by Gram's method.

Vital Activities.—It is a facultative aerobe, growing feebly in the absence of air, and best at 37° C., in glycerine agar. Resists drying but feebly. Its thermal death-point is 55° C., 10 minutes' exposure.

Chemical Activities.—Produces a brown pigment on potato, also mallein, and a little indol in old bouillon cultures. It forms no gas.

Cultures.—On **gelatine** it produces small punctiform colonies that are white, and become, after a time, surrounded by a distinct halo. The colonies are often very delicate and ragged. The gelatine is not liquefied. On **agar** the growth is best if glycerine is present, but is not characteristic. **Bouillon cultures** cause an abundant sediment, above which the media is clear. **Milk is coagulated.** On **potato** the growth is characteristic. The color is, at first, yellowish-white like honey, becoming, finally, reddish-brown. The potato is much darkened.

Pathogenesis.—This organism is pathogenic for horses and man;

fifty percent of men succumb after infection. Horses, asses, cats, dogs, sheep, and goats are susceptible in the order mentioned. Cattle and birds are immune. In horses the disease is known as glanders, or farcy, and the avenue of infection determines the clinical form of the disease. The mucous membrane and the skin are the chief places of infection. A primary ulcer is formed in the mucous membrane of the nose, or in the skin. Subsequently, the lymph glands and the lungs may be infected. Guinea pigs are easily infected. White and gray mice, and rats are immune. For purposes of diagnosis guinea pigs are inoculated, but care must be used, as several fatal cases have occurred in laboratory workers, it being a treacherous organism with which to work. In infected animals, it produces a rapid and marked inflammatory reaction, with the formation of pus. Certain "buds," or nodules are formed, which are between an abscess and a tubercle in structure.

Agglutinations.—It has been shown by McFadyean that the blood of infected horses exhibits markedly agglutinative properties towards the glanders bacilli.

Mallein.—In old cultures a peculiar tuberculin-like substance (mallein) is formed from the bodies of the bacilli themselves, and in the bouillon. This is thermostabile and if injected into animals having glanders, produces a marked reaction. Locally, if the horse is glanderous, a hard swelling is formed, and the temperature is raised 1.5° C. to 2° C. in a few hours. This is considered a valuable means of diagnosis by veterinarians.

DIPHTHERIA BACILLUS.

Corynebacterium Diphtheriæ. (Löffler.)

Bacillus Diphtheriæ.

Klebs-Löffler Bacillus.

Diphtheria Bacillus.

Morphology and Stains.—Long, bent, or curved bacilli of irregular contour, frequently clubbed or filiform at one or both ends;

which contain chromatophilic granules, and often exhibit true branching; have no spores or flagella, and are not motile. According to Westbrook, stained bacilli are of three types:—(1) granular, (containing the *Babes-Ernst* granules); (2) barred like a striped stocking; or (3) solid, staining uniformly throughout. The pleomorphic differences of various bacilli are most characteristic, and of diagnostic importance. This organism stains with all the basic dyes, notably by Löffler's blue, or Neisser's special granule

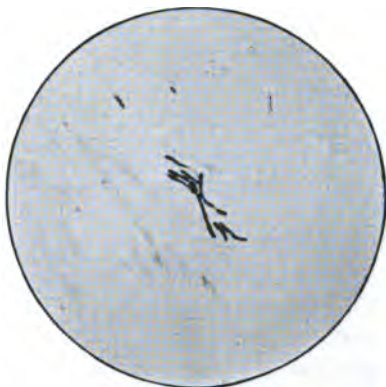


FIG. 43.—Diphtheria bacilli in mucus of trachea stained with fuchsin.
(Kolle and Wassermann.)

stain. It is also stained by Gram's method. The length of the organism differs much, according to the reaction of the media in which it grows. Alkaline media favors long forms, and acid the reverse. Its length is from 1.5μ to 3.5μ . It does not form chains. Bizarre, or involution shapes predominate in old cultures. (Fig. 44.)

Culture and Temperature Requirements.—It grows best at body temperature, and on glycerine agar, or in Löffler's blood serum mixture of alkaline reaction.

Vital Characteristics.—It resists drying for a long time, and has

lived on culture media for eighteen months at room temperature; also in silk threads for several months in a dried condition. Remains alive in healthy throats for months. Formalin vapor kills it speedily; corrosive sublimate solution, 1-10,000 destroys it in a few minutes; light is lethal to it in from two to ten hours, and heat at 58° C. in ten minutes.

Habitat.—It has not been found in sewage, or sewer gas, soil or water, the disease therefore is never transmitted by these means. Has

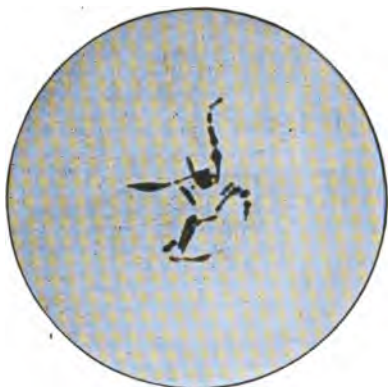


FIG. 44.—Diphtheria bacilli involution forms. (Kolle and Wassermann.)

been found in the throat, nose, and in the conjunctivæ of healthy bodies. In disease, the organism is mostly found in the throat, but has been isolated from all the organs in some fatal cases. Sometimes it is discovered in the throats of animals. Though its action is local, it elaborates a toxin which acts systematically.

Cultures.—On **gelatine plate** the growth is scanty and raised. This media is never used for cultivating this organism. The gelatine is not liquefied. On **glycerine agar plates** the growth, though moderate, is typically characteristic, but very slightly raised above the media, and is of duller lustre. Old colonies become yellowish-

brown, the center of which, under a magnification of sixty diameters, appears darker, and with ravelled edges. On Löffler's blood serum mixture, the organism grows rapidly and well. This and **ascites-glycerine-agar culture media** are the best for it. **Bouillon** made from fresh meat is an excellent media for its growth. The bouillon, which must be alkaline and freshly made, becomes first cloudy; then a fine precipitate settles, and over the surface a delicate pellicle forms. The reaction of the culture presents three types: A, is acid in the beginning, and becomes progressively more acid. B, is alkaline from the start, and progressively more alkaline; this is the most toxic growth. C, acid at the start, becoming alkaline finally. The growth is not so luxuriant as in B, nor is there as much toxin produced. In **milk**, the growth is luxuriant, without coagulation. The reaction is amphoteric, but in old cultures it is alkaline. On **potato**, rendered alkaline, it will grow, but not characteristically.

Chemical Activities.—No gas is formed, or any curdling or gelatine dissolving ferments. But H_2S , and indol, are produced. Acids are evolved from sugars, even the sugar found in meat is converted into lactic acid. In the manufacture of toxin, this muscle sugar must be removed. A soluble toxalbumin is created, both in the body and in culture, which is intensely poisonous. See chapter on bacterial products.

Pathogenesis.—Diphtheria in man means generally an infection of the mucous membrane of the upper respiratory tract, with the formation of false membranes. The latter may cause death by suffocation. Infection may occur in the skin, vulva, or prepuce. The toxin not only causes a local necrosis, with the formation of an exudate, consisting of fibrin and leucocytes, but also grave systemic action, with marked degeneration of important nerves and nerve centers, and also of the parenchyma of the kidneys, liver, and heart, paralysis following. In certain structures fragmentation of the nuclei of the cells is noted. Guinea pigs, cats, horses, and cows, may be infected artificially, but the disease never occurs spontaneously in these animals. Horses, dogs, and cattle are susceptible to its toxin.

Diphtheria bacilli often have associated with them streptococci, which adds to their virulence, and complicates the disease. Endocarditis, adenitis, pneumonia, abscesses, and empyemia, may be caused by them. There may be puerperal diphtheria, due to the infection of the puerperal tract. Diphtheria is spread mostly by personal contact with individuals suffering from the disease, or with convalescents, in whose throats virulent bacilli linger, perhaps, for months. It may originate from infected milk, contaminated from human sources.

Immunity is natural, active, artificial, or passive. Active immunity, following infection, is generally a permanency, for, once infected, the individual, if he recovers, may be considered immune, though some individuals are more susceptible, and suffer several attacks. In active immunity anti-toxin is found in the blood, and recovery, and subsequently, immunity is due to this fact. Anti-toxin may be discovered in the blood, by mixing it with toxin of known strength, and injecting it into guinea pigs. If these survive a large lethal dose of the toxin, it is safely presumed that anti-toxin was present in the serum abstracted.

Passive artificial immunity is induced by injecting anti-toxin in the bodies of persons exposed to diphtheria. It is most effective but is short lived, lasting only a few weeks. **Serum therapy** (see anti-toxin in previous chapter). If there is one natural specific cure for any disease, it is diphtheritic anti-toxic serum, which is prepared by immunizing horses with toxin, and abstracting their blood. This is measured in units, 1,000 to 5,000 units forming a dose. The earlier it is given, the better are the chances of recovery. As a prophylactic, from 600 to 1,000 units should be used. As many as 100,000 units have been injected in a single patient. No case is too trivial, or too far advanced in which to use it. The serum is anti-toxic, and not bactericidal. Wassermann has prepared a serum that is bactericidal, and is designed to destroy the bacilli.

Pseudo-diphtheria bacilli, which morphologically and culturally resemble the true bacilli, have been described. They are not

pathogenic, and are believed to be attenuated diphtheria bacilli by many observers. The diagnosis of diphtheria by culture is an important measure. It depends upon the rapid growth of the bacilli upon Löffler's blood serum in sterile test-tubes. Of all the various organisms found in the throats of patients with diphtheria, the diphtheria bacilli outstrip them in rapidity of growth. After eight to twelve hours, the serum inoculated with the smear from the false membrane is covered with fine granular colonies of pure diphtheria bacilli. After twenty-four, or more hours, the other organisms present overgrow the diphtheria colonies.

A sterile swab of cotton, or a stick, is rubbed over the false membrane, or throat, and then over the serum; the latter is incubated, and the culture examined after eight or twelve hours, by staining with Löffler's blue. If curved, clubbed, irregularly stained bacilli are found, especially if they contain dark polar granules, and are generally uneven in size and bizarre, it may be safely considered that they are diphtheria bacilli. Gram's stain may be needed to confirm the diagnosis occasionally, or it may be necessary to inoculate guinea pigs.



FIG. 45.—Diphtheria bacilli stained with Löffler's blue. Striped. (Greene's Medical Diagnosis.)

PSEUDO-DIPHTHERIA BACILLUS.

Corynebacterium Pseudo-diphtheriticum.

Pseudo-diphtheria Bacillus.

Morphology and Stains.—This bacillus resembles the diphtheria bacillus. The rods, however, are shorter and thicker; otherwise, it stains like the true bacillus, but not by Neisser's method.

Culture.—On **glycerine agar** the growth becomes diffuse, spreading from the line of inoculation in a grayish-yellowish pasty expanse. It grows well on **gelatine**. In **bouillon** it forms a denser and more luxuriant growth than the true bacillus, and produces alkali only.

Habitat.—It is found in healthy throats and conjunctivæ.

Pathogenesis.—It is absolutely non-pathogenic for man or guinea pigs.

Diagnosis.—It can be differentiated from the true bacillus by

1. Being non-pathogenic.
2. Not exhibiting polar granules with Neisser's stain.
3. Not producing acids in sugar bouillon.

TUBERCLE BACILLUS.

Mycobacterium Tuberculosis.

Bacillus Tuberculosis. (Fig. 46.)

Tubercle Bacillus.

Morphology and Stains.—Slender rods, generally unbranched, 1.5μ long, and $.4\mu$ thick, usually slightly bent; are non-motile, and have no spores or flagella. In old cultures, and sometimes in sputum, branching forms are seen, and, rarely, some that are club-shape.



FIG. 46.—Tubercle bacilli in sputum; stained with fuchsin and methylene blue. (Greene's Medical Diagnosis.)

On acid potato, thread forms are found. In the continuity of most of the bacilli, unstained vacuoles are seen; in others dense deep red granules, which are probably gonidia. As this bacillus is difficult to stain, special methods have been devised to demonstrate it, as the sheathing capsule renders it extremely unsusceptible to the ordinary methods of staining. The cause of this resistance is supposed to be due to a fatty or waxy substance in the capsule which is more than probable, because of the fact that stains that are fat selective, such as Sudan

III, color it very well. Boiling hot carbol-fuchsin gives it the best stain. It keeps the color in spite of the action of strong solutions of mineral acids in water, or dilute alcohol. So when tissues, or secretions, are stained with hot carbol-fuchsin for a short time, or cold carbol-

fuchsin for a long time, and then treated with a 25 percent solution of HNO_3 , or H_2SO_4 , in water, everything is deprived of the red color, except the tubercle bacilli. All such organisms that are acid proof, are called "acid-fast." There are many other bacilli that have this property. Aniline water and gentian violet solution also stain it. Gram's method dyes the organism violet. Sometimes very young bacilli do not stain at all.

Vital Requirements.—This bacillus thrives best at 37.5°C . It grows slowly, is a strict parasite, and an obligate aerobe. In cultures it dies quickly in sunlight, and in diffuse daylight it dies in a few days. It resists drying and light in sputum for months. Its thermal death-point (moist) is 80°C . for ten minutes; can resist 60°C . for one hour, but succumbs to 95°C . in one minute. It is quickly killed by formaline and corrosive sublimate, but resists 3 percent solution of carbolic acid for hours. In sputum it withstands antiseptics for a long time.

Chemical Activities.—It grows slowly, producing no coloring matter; yields an aromatic sweetish odor, but no gas or acid. It produces certain plasmins or endo-toxins, which are called tuberculins (q.v.).

Habitat.—It is a strict parasite and never leads a saprophytic existence. Is found wherever human beings live in crowded quarters; in dust of rooms, vehicles, and streets; and often in milk and butter. Has also been observed in the tissues and secretions of non-tuberculous persons. It is very widely distributed, being found in all human communities.

Cultures.—Since the organism does not grow below 30°C ., gelatine is never used. On coagulated **blood serum** of cows, horses, and dogs, this bacillus grows best. As it is very difficult to isolate in pure cultures, the following procedure should be followed: The suspected sputum, fluid, or tissue is injected into a guinea pig, and when, in two weeks or more, large swollen glands can be felt in the groin, the animal should be killed, and a gland removed under strict aseptic precautions. It is then divided, and the halves con-

taining the bacilli are rubbed over the surface of **coagulated dog serum** and allowed to remain in contact with it. The serum should be coagulated in special tubes, with glass caps, having small perforations, which are stopped with asbestos fiber, or glass wool. The organism grows well in air, but too great access thereto dries and kills it. After the tubes are incubated for a week or two, little scales growing into clumps appear, which are lobulated and friable. At first white, it later turns darker. This media is never liquefied by the culture. On **glycerine agar** made of veal broth containing 6 percent of glycerine, the organism grows well after isolation from the tissues, often luxuriantly. (Fig. 47.)

A wrinkled film covers the surface of the agar, from which it is removed with ease. On **bouillon**, made of veal and glycerinized, it develops rapidly, covering the media with a dense white wrinkled pellicle, which, though thick, is friable. After a time it falls to the bottom of the flask. It grows well on glycerinized potato also, and milk agar. On egg albumins mixed together, sterilized and coagulated, this bacillus also develops well.



FIG. 47.—*Bacillus tuberculosis*; glycerine agar-agar culture, several months old. (Curtis.)

Pathogenesis.—The discovery of the tubercle bacillus, its methods of cultivation and differential staining, may be ranked with the greatest of medical discoveries. This organism causes in man and cattle, chiefly, the disease called tuberculosis. It rarely attacks the carnivora, but has been found in such animals when confined. Swine are often infected; cats and dogs sometimes, but sheep, goats, and horses seldom. It is easy to inoculate guinea pigs or rabbits by injection or feeding. The disease is widespread, but is much more common where human beings are huddled together in dark, badly ventilated rooms and shops. In tissues, the characteristic lesion is a tubercle. This is a globular mass, about the size of a very small shot, and grayish pearly white. Microscopically, in the center of the tubercle, are found several large multinuclear cells, called giant cells, which often contain thirty or more nuclei, and a number of tubercle bacilli, the nuclei being often situated at one pole, while the bacilli are at the other. About the giant cells epithelial cells are grouped, and about these leucocytes (phagocytes) are massed in great numbers. No new blood vessel formation is ever found in the epithelial cell layers, or among the giant cells. Owing to insufficient blood supply the center of the tubercle frequently undergoes caseous degeneration. If the lesion heals, the caseous centers become calcareous, and the periphery changes into connective tissue. If the tubercles coalesce, great masses of caseous tissue form. If the latter becomes infected with other pathogenic bacteria (streptococci and pneumococci) rapid softening occurs, with cavity formation, etc. Tubercles may develop in any organ or tissue of the body. The lungs, intestines, peritoneum, glands, larynx, spleen, and bones become infected. The liver seems to resist invasion more than other organs. Bacilli are rarely found in the blood in tubercular diseases. They may, however, be found in the urine, and in kidney, or bladder tuberculosis. Milk from tuberculous cows, with infected udders, often contains bacilli, and is certainly a means of transmitting the disease. Cerebro-spinal fluid, in tubercular meningitis, often contains the bacilli. Bacilli may penetrate

mucous membranes, and not cause any local lesions, but infect distant organs. Tuberculosis may be spread in the body in four ways. Sputum may be swallowed and infect the intestines, or it may attack the larynx from the lungs. Infection may spread by continuity, by the lymph stream, or by the blood. Ingestion of bacilli may cause intestinal ulceration and invasion of the peritoneum, also the tonsils. If the bacilli reach the blood stream, the disease produced is generally acute miliary in type. This is manifested by the formation of fine gray tubercles. In tuberculosis of the lungs it is more than prob-



FIG. 48.—Tubercle bacilli showing involution forms. (Kolle and Wassermann.)

able that the bacilli are inhaled. Local tuberculosis has often followed skin inoculation, either by accidental or intentional trauma. Tuberculous mothers often have infected ovæ, and fathers, having tuberculous testes, discharge bacilli in the semen. Placental transmission of the bacilli from mother to child occurs.

Types of Tubercle Bacilli.—It has been considered probable by many observers that there are two types of bacilli, a human and a bovine type. Theobald Smith was the first to advance this theory.

Koch has announced that the two types were totally different, and that the human was incapable of infecting cattle, and the bovine was not pathogenic for man. In view of the fact that cattle are frequently tuberculous, and the bacilli are often found in the milk, it is important to know if the bovine type can develop in man. Ravenel has shown that it is undoubtedly pathogenic for human beings. Men have been infected in the hands, while performing autopsies on tubercular cattle, and their skin lesions showed, histologically, unmistakable tubercles. Cattle have been infected by bacilli of the human type. The bovine type of bacillus differs from the human in the following ways:—

1. It is much more pathogenic for guinea pigs and rabbits.
2. It produces more extensive lesions in cattle.
3. It is shorter than the human.
4. It produces more alkali in acid media.
5. It grows but feebly on media in which the human type grows well.

It is not always easy to differentiate the tubercle bacillus from other pathogenic and comparatively harmless acid-fast bacilli. Among these are the *B. Lepra*, the *B. Smegma*, and a number of organisms found in butter, milk, hay, grass, and in the blind worm. Culturally, the difference is great. "Tuberculins" (using the term as a convenience to describe extracts of cultures), of the different acid-fast bacilli, if injected into animals already infected with the same type of organism from which the extract was made, cause the animal to react towards the "tuberculin." If a tubercular cow was injected with a "tuberculin" of a grass bacillus, no reaction would occur, while a tubercle bacillus "tuberculin" would cause the reaction. This shows that the grass bacilli and the organism infecting the cow are not identical. We are able, in this roundabout way, to differentiate the various acid-fasts (Moeller). By using carbol fuchsin as a stain, and a twenty-five percent solution of H_2SO_4 as a decolorizer, and after allowing the latter to act for sixteen hours, it has been found that all of the "acid-fasts,"

except the tubercle bacilli, are decolorized, but this is not always reliable. The tubercle bacillus resists this acid solution seventy-two hours. By using a concentrated aqueous solution of methylene blue as a stain for ten minutes, at room temperature, the tubercle bacillus is not colored, while the smegma, timothy-hay, and lepra bacilli are well stained. The surest way to differentiate the tubercle bacillus from other acid-fast organisms is by animal inoculations.

Immunity.—It is possible to immunize cattle against virulent bovine tubercle bacilli by inoculating them previously with a culture of human tubercle bacilli that have been grown for some time on culture media, and then attenuated. The new tuberculins, if injected into a person with chronic tuberculosis, stimulate the development of anti-tuberculins, which act as a means of prevention or defense against further infection. Thus far anti-tubercular sera are not of a pronounced or certain therapeutic value. By immunizing horses, Maragliano obtained a serum that he claims is effective. The milk from immunized cattle is used as a diet in tuberculous patients by him. The various tuberculins, some containing endo-toxins, or plasmins, in solution, are capable of stimulating the formation of agglutinins in the sera of man and animals. Blood from infected individuals also contains these bodies. The *agglutination test* does not seem to be of great practical diagnostic value.

Note.—Tubercle bacilli causing avian and fish tuberculosis, and other acid-fast bacilli exist, but not being pathogenic for man, are not described here.

BACILLUS OF LEPROSY.

Mycobacterium Lepra. Hansen.

Lepa Bacillus.

An acid-fast organism resembling the tubercle bacillus morphologically when seen in secretions. Since very satisfactory cultures have not been obtained, it is difficult to describe its exact morphology. It is shorter than the tubercle bacillus, is non-motile, and

probably has no spores. In general it greatly resembles the tubercle bacillus, morphologically and tinctorially, though the granules are coarser and farther apart in the *B. Lepra*. Certain branched forms appear. The morphology, at times, is like the diphtheria bacillus. It stains by Gram's method, also by carbolfuchsin. It is acid-fast, but does not resist the action of acids nearly so well as the tubercle bacillus.

Cultures have been made on serum and glycerine agar, which, though resembling the tubercle bacillus, are more delicate, and not so luxuriant. It grows best in the total absence of sodium chloride.

Pathogenesis.—It is highly pathogenic for man but not for animals, producing in the former a slow chronic disease, which is, probably, transmitted by more or less intimate personal contact. The bacillus is seen in enormous numbers in lepra cells and elsewhere in diseased tissues and has been found in the blood. The lepra cells are larger and vacuolated, and literally crammed full to bursting with bacilli. In general the leprous lesion resembles a tubercle, as it consists of giant cells, epithelial, and round cells.

Immunity.—There is very little accurate knowledge as to immunity against this organism.

RAY FUNGUS.

Actinomyces Bovis.

Ray Fungus.

Morphology and Stains.—This organism is called the ray fungus because of the stellate arrangement of its threads in the colonies found in tissues. It is of a more complex structure than the bacteria hitherto described. There are three elements found in every colony: 1. Long threads which may be branched or unbranched. 2. Threads that are clubbed, which may, or may not, be branched. 3. Spore-like bodies contained within the threads, from the ends of which they are discharged. The colonies in tissue are often 1 mm.

in diameter, and made up of many clubbed-shaped threads radially situated. Through the periphery and extending beyond are other unclubbed threads, while scattered throughout the colony and beyond it, and in the threads, may be seen many spore-like bodies. The threads and spores stain by Gram's method, while the clubs do not. Basic stains also color all the elements. The spores do not stain like bacterial endo-spores.

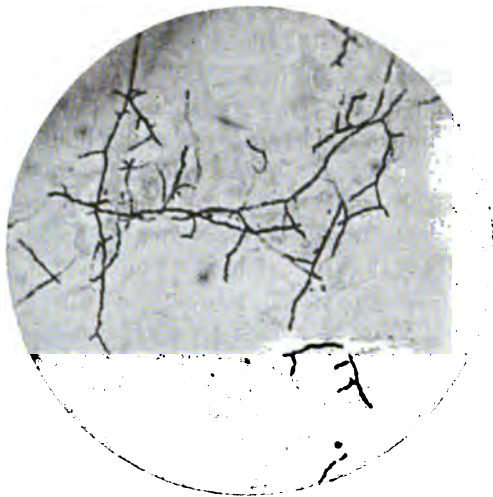


FIG.—49.—Actinomycosis bovis. (Williams.)

Vital Requirements.—It is a facultative aerobe, and grows best in the presence of air, at 37° C. Resists drying for a long time, and its thermal death-point is 80° C. after fifteen minutes, exposure.

Chemical Activities.—Slowly liquefies gelatine, does not curdle milk; and produces a mouldy odor. No gas or acids are formed, nor is H_2S developed.

Habitat.—It has been found in straw and hay, but never in a healthy body.

Cultures.—On **gelatine plates** it produces yellowish-gray colonies that are very small. These grow into the gelatine, slowly liquefying it. The colonies are very tough and fibrous. In **agar tubes** it grows very slowly, the first growth being like dew-drops; later these enlarge, turning yellow, and finally brown. The culture grows down into the agar, and the media darkens. Old cultures are dark and crumbly looking, adhere firmly to the agar, and have a downy dust-like covering. On **blood serum** the colonies appear as dew-drops, which later become brownish, then, yellowish-orange, or brick-red. In **bouillon** the growth is at the bottom in ball-like masses, that firmly cohere. Clubs do not form in this media. The supernatant bouillon is clear, with no surface growth. In **milk** it produces no chemical change. On **potato** it grows in knot-like colonies.

Pathogenesis.—Causes in cattle the disease known as “lumpy jaw.” The fungus reaches the jaw from the teeth and gums, the latter first being injured by sharp spines in the food. In man, the internal organs, lungs, intestines, and, rarely, the brain become infected. The liver often is abscessed. In both cattle and man universal actinomycosis sometimes occurs. It is hard to inoculate laboratory animals with the disease, though Wright succeeded in so doing. The lesions produced are rather massive at times; the nidus is often surrounded by enormous numbers of polynuclear leucocytes, which, no doubt, play a defensive role in the tissues. The disease is often fatal to cattle and to man.

FARCIN DU BOEUF.

Actinomyces Farcinicus.

Bacillus du farcin du Boeuf. Nocard.

Morphology and Stains.—Segmented threads with true branching, short and knotty, or long and delicate. Contains spores, is not motile,

and has no flagella. It stains with all the ordinary aniline dyes, and by Weigert-Gram method. Ziehl's method stains it well. It is often seen as tangled masses of threads.

Vital Requirements.—Grows well at room temperature, and in the incubator. Nocard kept a culture at 40° C. for four months and it was still virulent.

Cultures.—It thrives well on all culture media. On **bouillon** the growth is colorless, and in masses that float and then sink; or in a fenestrated pellicle on the top. On **Agar**.—It appears in discrete little roundish yellowish-white masses that resemble lichens. On **blood serum** its growth is like that on agar, only less luxuriant. On **potato** it is scaly, wrinkled, yellowish and dry. In **milk** the organism flourishes, without curdling the milk or altering its reaction.

Pathogenesis.—This organism is pathogenic for all the laboratory animals. Sheep, dogs, wild rabbits, horses, asses, and men are immune. It produces abscess, in those animals for which it is pathogenic, that discharges, with subsequent induration, ulceration, and sloughing. The disease in cattle resembles glanders.

If injected into the blood, miliary tubercles are found that resemble tuberculosis.

ACTINOMYCES MADURA.

Actinomyces Madura.

Streptothrix Madura, Vincent.

Morphology and Stains.—A non-motile, non-flagellated organism said to have spores. Its growth resembles that of *actinomyces bovis*. It consists of long threads that are clubbed. These stain by all the basic aniline dyes and by Gram's method.

Vital Requirements.—It is a facultative aerobe. The thermal death-point for the spores is 85° C. for three minutes, and 75° C. for five minutes. Vegetative thread forms die at 60° C. Grows best at 37° C., and scantily at room temperature.

Cultures.—Generates upon all culture media. In **Bouillon**.—It

appears in little clumps which cling to the glass, and are bright red in color, eventually they sink to the bottom in pale masses. **In Gelatine.**—It grows sparingly in clumps, slowly liquefying the media. **Upon Agar.**—It forms shiny round colonies, that are first devoid of color, then become deep red. They resemble an umbilicated vaccine vesicle and adhere tightly to the agar. **In Milk.**—It grows without coagulating the medium. **On Potato.**—The culture

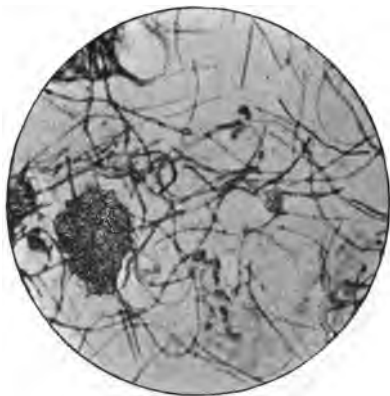


FIG. 50.—*Streptothrix hominis*. (Kolle and Wassermann.)

is very slow, and without chromogenesis. Old colonies are powdery, due to spores.

Pathogenesis.—In man it produces madura foot, an affection characterised by induration, ulceration, and fistulæ formation with pus.

STREPTOTHRIX (Eppinger).

Are organisms that probably belong to the actinomyces. Kruse has described nineteen different members of the streptothrix, some pathogenic to man and animals.

Lately a number of cases of streptothrix (*Streptothrix Hominis*)

infection in man have been reported. The disease, in general, resembles phthisis. In the pus, sputum, and stained sections of these cases, streptothrichal threads have been found. (Fig. 50.)

Morphology and Stains.—Threads are thick and short, or long and slender, depending upon the medium on which they grow. In **bouillon** the threads are thin and long, on **blood serum**, short and thick. When stained there is distinct beading and fragmentation of the protoplasm.

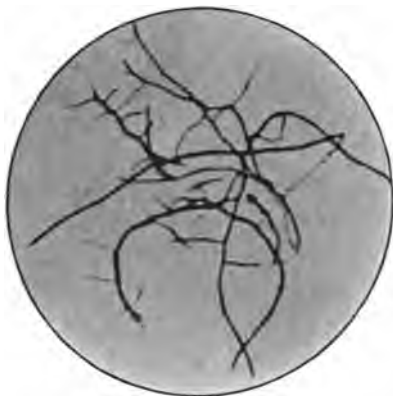


FIG. 51.—*Streptothrix candida*. (Kolle and Wassermann.)

There is true branching of an irregular type, which is best seen in liquid media. These threads often produce spores on culture media. The threads often disappear in old cultures, leaving only the spores, which stain with carbol-fuchsin and do not decolorize. The threads stain by Gram's method, and Gram-Weigert method. The threads are not acid-fast.

Vital Characteristics.—These organisms live for years in culture media after it is dry. Spores resist dry heat at 60° C.—70° C. for an hour; moist heat, 60° C. however, kills them after an hour. It is a strict aerobe.

Cultures.—On **Löffler's blood serum**, according to Tuttle, this

organism grows slowly in whitish colonies, which finally become yellow. The adult colonies adhere to the serum. **On Agar** it grows rapidly and characteristically. The colonies are yellowish-white and adhere to the agar. **In Bouillon**.—It develops slowly on the surface of the media. Fluffy tufts, or balls, are formed, that sink to the bottom of the tube. The growth is whitish.

Pathogenesis.—For rabbits and guinea pigs this organism is pathogenic, producing abscesses, tubercles, induration, etc. It is a pus forming organism.

In man, the disease picture is like that of tuberculosis. It causes abscesses, adenitis, indurations of the skin, endocarditis, and pleuritic inflammation. Many grayish tubercles were found that resembled the lesions produced by the tubercle bacillus. Cavity formation has been described.

This organism acts as a secondary infecting agent in tuberculosis of the lungs. Tuttle reviews twelve cases, all of which were fatal.

In examining sputum from tubercular cases, in which the typical bacilli are not found, it is well to look for the streptothrix by staining with Gram's stain.

Leptothrix Buccalis.—Long unbranched threads that grow in the walls of the pharynx, causing very sore throat. This organism has not been cultivated, hence, very little is known of it. It is not a member of the actinomyces, because it is not branched, nor is it a streptothrix for the same reason.

Leptothrix Vaginalis.—Is another variety that has been found growing in the vagina. Nothing is known of its pathogenicity, nor of its cultural properties.

OIDIOMYCOSIS.

Oidium Albicals. *Thrush, Soor*.—A member of the higher order of the fungi. This organism resembles both a yeast and a mycelium, because it exhibits characteristics that are common to both of these two forms. It exhibits budding yeast cells and bud-

ding mycelia. The yeast cell is $6\ \mu$ long and $1\ \mu$ wide, but the cells vary very much in length and width.

It stains well in tissues and cultures by Gram's method, and by the ordinary basic stains. It may be cultivated on bouillon, blood serum, agar, potato, etc., and it is rather indifferent to the reaction of the media. It grows best if sugars are present. It is, however, very susceptible to such antiseptics as phenol, salicylic acid, sublimate, etc.



FIG. 52.—Thrush fungus. (Kolle and Wassermann.)

Pathogenesis.—Causes in man a condition known as oidiomycosis, and in young children a very troublesome stomatitis, which, if the child is weak and illy nourished, may result seriously. It may cause metastatic abscesses in the brain, spleen, and kidneys, or nodules in the lungs. This organism may penetrate mucous tissues, and fill the lumen of vessels (Virchow). By repeated injections of cultures into rabbits anti-oidium serum may be prepared. This serum exercises a bacteriolytic and an agglutinative action on the oidium which normal serum does not have.

Oidium Coccidioides, Ophuls. (Blastomycetes).—In and near Chicago there have appeared parasitic inflammations of the skin

that have been termed blastomycetic dermatitis. From the lesions of this disease fungi have been cultivated which resemble closely the blastomycetes, but Ricketts and Ophuls have placed this organism in the *oidium* genus. Not only does it cause an infectious dermatitis, but it may invade the deeper tissues and organs. The lungs may be primarily invaded, setting up in them an oidiomycosis that resembles or imitates in its general appearance pulmonary tuberculosis. The *oidium* may be detected in the sputum, and exhibits budding. It is easily stained.

Saccharomyces Bussi.—A pathogenic yeast that invaded the tissues of a woman, setting up broken-down nodular lesions in the kidneys, lungs, and bones, causing death. There are other members of the yeast family that are the cause of pathogenic processes in man.

Aspergillus, Niger, A. Fumigata, and A. Flavus.—A poly-cellular mycelial organism which produces spores and branched threads that stain, that are variously named from the macroscopic appearances of the growth. All thrive well at 37° C. and may be cultivated on the usual culture media. In man, the external auditory meatus is often infected with these organisms, causing a troublesome disease. It may infect the lungs of weak anemic subjects with wasting diseases, and is pathogenic for cattle, horses, and birds.

The author has found that the young hyphæ, the sporangia, and spores of some of these hyphomycetes (moulds) if treated with hot or boiling alkaline solution of copper sulphate are stained by the copper, which has an affinity for them, and appear a light lilac blue under the microscope. If treated with a solution of ferro cyanide of potash and acetic acid, these stained parts turn a dark brown, showing that there is an actual absorption or perhaps chemical union of the protoplasm of the mould with the copper. Some moulds are stained a deep blue, and are visible to the naked eye in test-tubes, after treatment with the boiling alkaline copper others are colored a bright yellow. Some moulds and bacteria have the power of reducing copper in Fehling's solution.

CHAPTER VI.

ANIMAL PARASITES.

While numerous diseases are caused by vegetable parasites, such as bacteria and moulds. there are others in which the etiological rôle is played by minute microscopic organisms of the animal kingdom. There are also infectious diseases that are supposedly caused by animal parasites, and yet, the exact knowledge that they are the cause is lacking. In only one instance can the postulates of Koch be fulfilled by an animal parasite, and that is in the case of one of the trypanosomes, which can be grown on culture media, and infection of animals, in turn, produced by the cultures.

In general, it may be said of animal parasites, particularly those belonging to the protozoa, that an intermediate host, such as a suctorial insect, is necessary for the transmission of the organism to man or animal. This is called alternate generation and is a very characteristic feature.

The protozoa, as parasites in man, are the cause of several well-known diseases, namely:—Dysentery, malaria, sleeping-sickness, and coccidiosis. In hydrophobia, scarlet fever, and small-pox certain peculiar bodies are constantly found that resemble protozoa, but since it is not known whether they are animal bodies at all, they cannot be classed as protozoa, though they will be described as such.

PROTOZOA.

The protozoa are divided by naturalists into five classes:

- I. Rhizopoda.
- II. Flagellata.
- III. Sporozoa.
- IV. Infusoria.
- V. Suctoria.

The protozoa are always, in every stage of development, primitive unicellular bodies. They consist essentially of a cell-body or *sarcode*, a *nucleus*, and a *nucleolus*. All of the vital functions of the cell are carried out by the cell-body, the protoplasm of which digests and assimilates food. Particular parts of the protoplasm have special functions, these parts are called *organelles*. The living protoplasm is finely granular, is viscid, and exhibits a distinct movement; the pseudopoda are projected and withdrawn, and the animal is thus able to move from place to place. In most protozoa two layers can be seen—the *ectosarc*, and *endosarc*. The *ectosarc* originates the movement, is concerned in the ingestion and excretion of food, and the respiration. The *endosarc*, which circulates slowly, is mainly for digestive purposes. In it are ferments, crystals, food particles (seen in the *food vacuoles*), oil globules, gas, and pigment granules.

Flagella and suctorial tubes—in protozoa that have them—belong to the *ectosarc*. Skeletal tissues, shells, etc., also belong to this layer.

The food consists of bacteria, smaller animals, algæ, and animal waste.

Propagation is effected by direct cell division, beginning in the nucleus, or by cell budding. Sometimes division, or budding, occurs rapidly without the segments separating, leading to the formation of protozoa colonies, or swarm spores.

In the case of the malarial plasmodia, asexual development, (*schizogony*) takes place in man's blood, while the sexual development (*sporogony*) takes place in the mosquito. Protozoa are found in salt and fresh water, in damp places, and in animals as parasites. There are but two Rhizopods that are parasitic and pathogenic to man. The only one of these of any import is the Amœba.

AMCEBA DYSENTERIÆ.

This is a pear-shaped roundish body from .008 to .05 mm. in diameter. The *ectosarc* is easily discernible in the pseudopodia,

but not in the round quiescent cell. In the endosarc, which is granular, vacuoles are easily seen; so are fragments of food, red and white blood cells, bacteria, epithelial cells, and fecal matter. The pseudopodia are broad and lobose; one or two are protruded at a time. The motion of the organism depends upon the reaction of the media, and the temperature. The vacuoles and nucleus are always present. Propagation generally takes place by binary division, the process beginning in the nucleus. When irritated, the amoeba at once assumes a spherical form, the pseudopodia being withdrawn.



FIG. 53.—Amœba dysenteriae. (Greene's Medical Diagnosis.)

Habitat.—They have frequently been found in the alvine discharges of healthy men and animals. They no doubt exist in water.

Pathogenesis.—Amœba Dysenteriae is not always pathogenic. But Calandruccio succeeded in setting up a dysentery in himself by swallowing amoeba. While considered to be the cause of dysentery, yet this cannot be proved absolutely, because pure bacteria-free organisms cannot be obtained, and the postulates of Koch cannot be fulfilled. In the lower gut of man and cats, in dysentery cases, encysted amoeba are often found. They have been seen in the liver (in old cases), also in the lungs and sputum. In over 500 cases of dysentery the amoeba was present in every instance.

Cats have been infected by pus from liver abscesses devoid of bacteria (Kastules). The urine, in cases of cystitis, contained amoeba, and it is believed to be the cause of the disease in some rare instances. In dysentery the amoeba are the cause of the necrosis and ulceration, as they frequently become encysted in the submucous tissues. From the

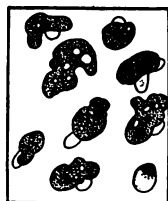


FIG. 54.—Ameboid motion. (Greene's Medical Diagnosis.)

amœba coli the dysenteric *amœba* is differentiated by the fact that it is larger, coarser in structure, and takes up red blood cells, which the former does not.

In stools (from dysenteric cases) over a day old, *amœba* are not often found, as they undergo a rapid disintegration outside the body.

FLAGELLATA.

The flagellata derive their name from the fact that all are possessed, at some time in their existence, of flagella, which are not only organs of locomotion, but serve to apprehend food.

The principal members of this class of interest from a pathological view-point, are the trypanosomes. The **Trypanosoma Brucei**, which causes the tsetse-fly disease (*nagana*) in horses and cattle, is transmitted to cattle by the bite of the tsetse-fly (*glossinia morsitans*). It can be grown on blood agar (Novy).



FIG. 55.—Flagellata. (Greene's Medical Diagnosis.)

Trypanosoma Evansi causes surra, a disease of horses in Central Asia.

Trypanosoma Equiperdum causes a sexual disease in stallions and mares called dourine; this is akin to syphilis in man.

Trypanosoma Lewisi of rats is transmitted from animal to animal by means of fleas.

Trypanosoma Noctuæ.—A parasite of the little owl, which is introduced into the bird through the bite of the mosquito *culex pipiens*.

Trypanosoma Carassii are elongated fusiform bodies pointed at both ends, provided by a fin fold, or undulating membrane, running along the dorsal edge and forming frill-like folds which terminate in a whip-like extremity or flagellum.

A large nucleus is always seen, also a centrosome, a small chromatic mass—likewise called a blepharoplast—near one pole.

The flagellum is at the anterior extremity; the short pointed end is the posterior extremity. Cell division begins in the nucleus, the cell dividing longitudinally, the flagellum, centrosome, as well as the nucleus undergoing division. Trypanosomes frequently appear in clumps with the ends united, resembling a wheel.

The trypanosomes exist in two hosts—one a suctorial insect—and have a sexual and an asexual existence (alternate generation).



FIG. 56.—Trypanosome in rats' blood. (Williams.)

In an infected owl the organism has been observed clinging fast to the red cells, absorbing nutriment during the day, while at night it swims about freely in the plasma.

In owl's blood the trypanosome assumes asexual forms, called *macrogametes*. These macrogametes penetrate the erythrocytes, accumulating the remnants of the red cells in the protoplasm. The

nucleus of the trypanosome may be seen in the interior of the protoplasm. The microgametocytes arise from the asexual forms and when mature, give rise to eight microgametes.

TRYPANOSOMA GAMBIENSIS.

Castellani found that this trypanosome is the cause of sleeping disease amongst the natives of South Africa. The organism has been found in the cerebro-spinal fluid—in cases of sleeping-sickness—quite uniformly. They have also been found in the blood. The disease has a long period of incubation (months), runs a long course



FIG. 57.—Trypanosomes; showing ordinary structural appearance on left; in middle a trypanosome undergoing division; on the right an agglutinated group. (Tyson's Practice.)

usually, and, at its full development, it is a meningo-encephalomyelitis. This is characterized by hebetude, somnolence, and coma. These symptoms are accompanied by disturbance of the motor apparatus, oedema, irregular temperature, rapid pulse, emaciation, skin eruptions, and death in coma. In these cases the parasites may be seen in the blood slowly winding their way through the corpuscles. The pathogenic action is due no doubt to some toxin elaborated.

The disease is transmitted from man to man by the tsetse-fly (*Glossina palpalis*). In the fly it exists as a true parasite in a host, and not merely passively.

The disease does not depend upon the age, sex of the individual, nor upon drinking water, food, seasons, etc.

The organism may be stained by the ordinary blood stains, mixtures, such as Leishman's, Romanowsky's, etc., the nucleus, centrosome and flagella, staining deepest. Thus far the *T. Gambiensi*s has not been cultivated in artificial media.

Novy has succeeded in growing the *T. Lewisii* and *T. Brucei* on agar mixed with defibrinated rabbit's blood. These are the first animal parasites to be cultivated artificially.

In Dum Dum fever, a disease occurring in India, curious bodies, called Leishman-Donovan bodies have been found. These resemble the malarial plasmodia roughly, and if cultivated on blood agar trypanosomes develop. These bodies are evidently in the halteridium stage of trypanosome existence.

TREPONEMA PALLIDA (*Schaudinn*).

(Spirochæte Pallida.)

Morphology and Stains.—Most probably a member of the trypanosomes, extremely delicate in structure, from 4 to 14 μ in length and about .5 μ in width; has from 3 to 12 turns or bends, and its ends are delicately pointed. There is a suggestion of an undulating membrane, but none of flagella or spores. Some observers have detected a nucleus in it. As this organism is stained with difficulty it requires a special one, that of Giemsa yielding the best results. Aniline, gentian violet, Romanowsky's, and Leishman's stains also color it. It may be stained in tissues by silver and pyrogallic acid methods.

Habitat.—It has not been found in tissues of normal persons, or

those ill with carcinoma, tuberculosis, etc., but only in the tissues of individuals suffering with syphilis. It is a strict parasite.

Vitality.—Nothing is known of its ability to withstand the action

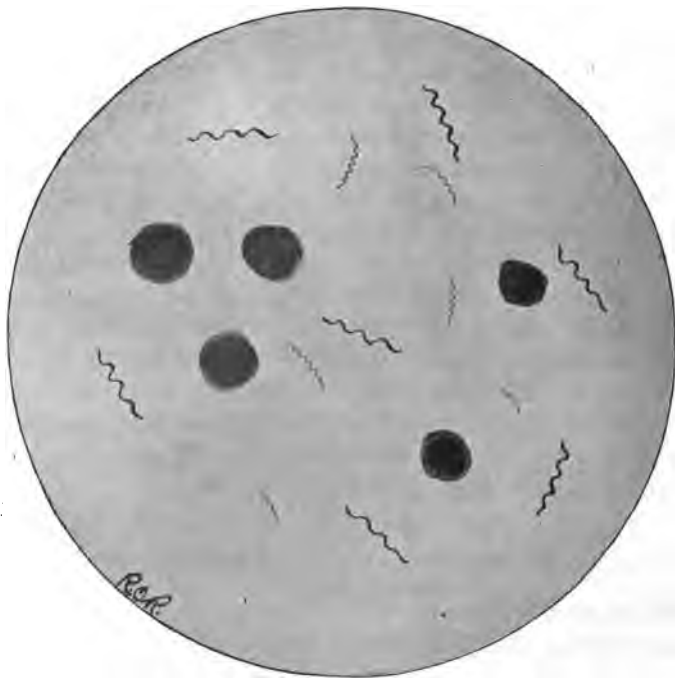


FIG. 58.—The spirochæta refringens is the larger and more darkly stained organism, while the lightly stained and more delicate parasite is the spirochæta pallida (*treponema pallidum*). From a chancre stained with Wright's blood stain. (Hirsch—by Rosenberger.)

of chemicals, light, heat, or other deleterious agencies. Glycerine destroys its motility.

Cultures.—None have been made.

Pathogenesis.—It has been found in chancre, condylomata, and

mucous patches in the early stages of syphilis; also in the blood, blister-fluids, spleen, bone marrow, liver, thymus gland, and lymphatic glands. Investigators claim that it exists in smegma and other foul secretions, but this has not been confirmed. Associated with this organism, in nearly every case, is a coarser looking larger spirochæte (treponema), which stains deeper, and has been called the **spirochæte (treponema) refringens**.

Metchnikoff believes that syphilis is due to infection with the *Treponema pallida*, which not only causes the disease in man, but in monkeys and apes by inoculation. In a series of very recent experiments, Metchnikoff and Roux caused abortion of the chancre following inoculation of syphilitic virus on the eyelid of a chimpanzee, by mercurial inunction carried out less than one hour after the infection; a solution of sublimate has not the same prophylactic property. Since it has not, thus far, been cultivated on artificial media, nothing can be said in relation to immunity, the postulates of Koch, etc. There are several reasons for thinking that it may prove to be one of the protozoa. It, of course, does not require any intermediate host for transmission as do the recognized animal parasites, of malaria and filariasis, etc.

RELAPSING FEVER ORGANISM.

Spirillum Obermeieri.

Spirillum of Relapsing Fever. (Fig. 59.)

Recently this organism has been considered to belong to the flagellate (Schaudinn) since it strongly resembles the trypanosomes and in its life history it presents several characteristics of the animal parasites, i.e., it cannot be cultivated on artificial media; infection probably follows the bite of some suctorial insect; only during a paroxysm of fever is it found in the blood, and disappears in the apyrexial period. Its motions and appearances resemble those of the trypanosomes. On the other hand it divides by direct division; there is also no evidence of a nucleus or blepharoplast; and

the absence of plasmolysis in distilled water indicates a bacterial relationship.

Morphology and Stains.—It is a long flexible spiral organism with delicately pointed ends, 16μ to 42μ in length, and about half as thick as the cholera spirillum. It has no demonstrable organs of locomotion, or internal structure, but is actively motile. Does not stain by Gram's method, but stained by Romanowsky's, Wright's and Jenner's methods and Löffler's blue.



FIG. 59.—Spirilla of relapsing fever from blood of a man.
(Kolle and Wassermann.)

If kept in normal salt solution they are actively motile for some time; 60° C. kills them, but they will survive 0° C. for some days. In glass tubes it remains alive for fifteen days.

Cultures.—Thus far it has been impossible to cultivate them on culture media of any kind.

Animal Inoculation.—Animals and white rats are very susceptible and the organism has been kept alive by passing it through a series of white rats for two months.

Blood from a spirochetal case, diluted with citrate salt solution may be forced through a Berkefeld filter and injected into animals,

producing in them typical spirochetal infection. This shows that the organism sometimes is submicroscopic.

Pathogenic to man. This organism is the cause of relapsing fever in man, and during a paroxysm it may be recovered from the blood. During the apyrexial period it disappears therefrom. The blood of an infected individual, injected into a healthy man, has produced the disease in him. It is more than probable that the disease is spread by some suctorial insect. The periods of fever last from five to seven days, when a crisis occurs. After an apyrexial period the fever recurs. It is found in great numbers in every microscopical field.

In the apyrexial period the spleen becomes engorged and the leucocytes devour the parasites. Monkeys with excised spleens are more susceptible to infection than others.

Immunity.—The blood from rats that have been immunized by repeated injections of blood from spirochetal rats, if injected into other rats, is capable of conferring an immunity on them by causing spirohætes to disappear from their blood.

SPOROZOA.

The most important of this family are the malarial parasites (which belong to the order Hæmosporidia), and the Coccidia.

In general the sporozoa are invariably unicellular organisms that lead a parasitic existence in the tissues of higher animals. They ingest liquid food, have no cilia in the adult stage, and flagella are possessed only by the males. There may be one or more nuclei. Propagation is effected by spores, but budding and division do occur, though rarely. Alternate generation takes place frequently.

Malarial Parasites.

Hæmosporidia of man. The disease caused in human beings by the hæmosporidia is malaria, or ague, and excepting the deserts, mountains, and arctic regions, this disease is very widely distributed.

Three different parasites producing different clinical entities are known. According to the time, frequency, and order of the outbreak of chills and fever, various clinical names have been given to the manifestation of the disease. Mannaberg has arranged the following scheme to show the different forms of outbreaks. The numbers apply to the paroxysms. Each developmental cycle is numbered alike:—

I I I I I I I.	<i>Simple quotidian fever.</i>
I O I O I O I.	<i>Simple tertian fever.</i>
I O O I O O I O O I.	<i>Simple quartan fever.</i>
I 2 I 2 I 2 I 2.	<i>Double tertian fever. (Two injections.)</i>
I 2 3 I 2 3 I 2 3.	<i>Triple quartan fever. (Three injections.)</i>
I 2 O I 2 O I 2 O.	<i>Double quartan fever. (Two injections.)</i>

The figures refer to days on which paroxysms of fever occur. The o represents the afebrile day.

PLASMODIUM MALARIE (Laveran).

This is the quartan parasite, and produces in man, in cases of one infection, paroxysms of fever every fourth day.

It appears in the blood, after a paroxysm, as a small non-pigmented body on the bodies of the red blood cells. It has feeble amœboid motion; slowly penetrates the corpuscle, and blocks of melanin appear in its protoplasm. Forty-eight hours after the attack the parasite measures from one-half to two-thirds the size of the red cell. Sixty hours after the paroxysm—twelve before the next—the parasite completely fills the red cell, leaving only a narrow rim, which later on disappears. Six hours before the next paroxysm, schizogony begins. The grains of melanin are arranged like the spokes of a wheel, and then, leaving the radii, crowd about the center (the rest of the cell being pigmentless) gradually dividing into nine or twelve pear-shaped bodies, or *merozoites*. These separate from each other and individually attack a fresh red cell, and this attack

brings about another paroxysm of fever seventy-two hours after the previous one. The grains of pigment are taken up by the leucocytes, and deposited in the spleen and bone marrow.

The nucleus of the parasite may be seen if suitably stained. The double or triple quartan is explained by the fact that there are two or three groups of organisms that undergo sporogony at periods separated from each by twenty-four hours.

PLASMODIUM VIVAX *Grassi*.

The cause of tertian fever occurring in the spring. It differs from the plasmodium malarie because of shorter period (forty-eight hours) consumed in schizogony (or sporulation), the much greater activity of the amœboid movement, and the affected corpuscles becoming enlarged. Also by the fact that many of the melanin-bearing stages are visible. The schizogony are rarely apparent in the circulating blood, but in the spleen these stages are easily seen. There are from fifteen to twenty merozoites (segmented bodies or spores) which are arranged in an irregular heap, but not radially like wheel spokes. The merozoites are smaller than the quartan variety and are more numerous. The flagellated form can but rarely be seen in the freshly drawn blood. If some, containing the large extra-corpuscular bodies, are put in a moist chamber, they throw out flagella. These flagella are really *microgametes* and are sexually active. The extra-corpuscular bodies are partly *macrogametes*, and if they become flagellated they are called polymites, and are the *microgametocytes*. The merozoites or spores, finally burst forth from the erythrocytes, starting again another cycle (attended with a paroxysm of fever). These spores appear in the freshly invaded corpuscles as hyaline bodies with slight movement. As they grow in size, pigment appears in the protoplasm. Certain of these do not break up into merozoites, or spores, but become extra-cellular bodies and polymites if they develop flagella in the moist chamber. There may be two infections in which schizogony occurs every other day in alternate days 1 2 1 2 1 2 1 2.

PLASMODIUM SP.*

The plasmodium of æstivo-autumnal fever, or pernicious malarial fever, also called tropical. The outbreaks of this occur irregularly. The disease produced by them is very much more malignant and is harder to cure. The young spore appears in the corpuscle as a small hyaline body, smaller than the other forms and much more active. The invaded corpuscle is apt to be shrunken, dark, and crenated. The pigment is very finely granular and the body frequently presents the signet ring appearance. The cycle of development (schizogony) is about forty-eight hours. The plasmodium in its schizogony divides into 7-12 merozoites or spores, and are arranged in a spoke-like form. The extra-corpuscular bodies may resemble a crescent or sickle; this form is very characteristic of æstivo-autumnal fever. They are very resistant to quinine and persist for a long period in the blood. Plasmodia undergoing schizogony are often found in the brain capillaries after death, which accounts for the cerebral symptoms in such cases. This form can be differentiated from the others by the irregular and pernicious type of fever produced; by its great resistance to quinine; the fewer number of merozoites; the finely granular appearance of the pigment; the relatively small size of the young intra-corpuscular body; and, by the ring shape of some of the young forms.

Often, in blood from malarial cases, pigmented leucocytes are seen, and ghost, or shadow, red corpuscles from which the hæmoglobin has been dissolved are often met with. Spherical extra-corpuscular bodies become flagellated (polymites) in freshly drawn blood. The parasite may be studied in fresh film preparations, and by staining dried films by methylene blue and eosin, Romanowsky's, or Jenner's methods. They are much more frequent in the pyrexial period, and when quinine has not been given.

The various plasmodia are transmitted to man invariably by the anopheles mosquito, in the bodies of which they undergo a different

*No specific name has been decided upon.

DESCRIPTION OF FIG. 6o.

Life history of malaria parasite, *Plasmodium*. 1, Sporozoite, introduced by mosquito into human blood, the sporozoite becomes a schizont; 2, young schizont; 3, young schizont in a red blood corpuscle; 4, full-grown schizont; 5, nuclear division; 6, spores, or merozoites, from a single mother-cell; 7, young macrogamete (female), from a merozoite, and situated in a red blood corpuscle; 7a, young microgametoblast (male); 8, full-grown macrogamete; 8a, full-grown microgametoblast; 9, mature macrogamete; 9a, mature microgametoblast; 9b, resting cell, bearing six flagellate microgametes (male); 10, fertilization of a macrogamete by a motile microgamete; the macrogamete next becomes an oökinete; 11, oökinete, or wandering cell, which penetrates into the wall of the stomach of the mosquito; 12, oökinete in the outer region of the wall of the stomach, i. e., next to the body cavity; 13, young oöcyst, derived from the oökinete; 14, oöcyst, containing sporoblasts, which develop into sporozoites; 15, older oöcyst; 16, mature oöcysts, containing sporozoites; 17, transverse section of salivary gland of an *Anopheles* mosquito, showing sporozoites of the malaria parasite in the gland cells surrounding the central canal.

1-6 illustrate *schizogony* (asexual production of spores); 7-16, *sporogony* (sexual production of spores).

(FOLSOM—After GRASSI and LEUCKART, by permission of Dr. Carl Chun.)

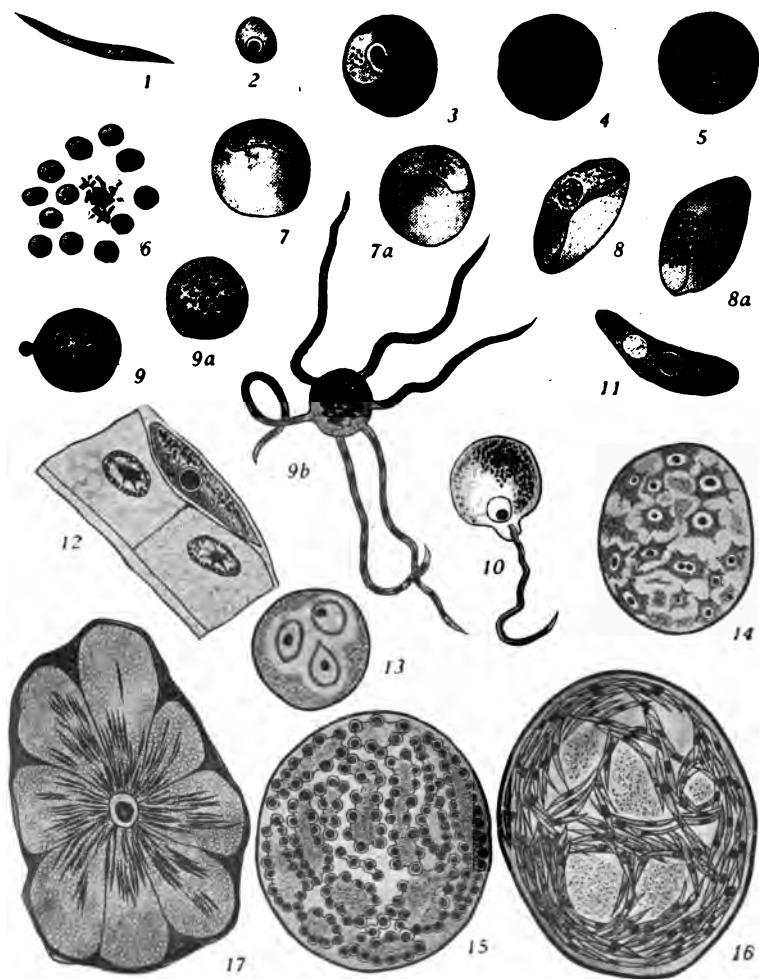


FIG. 60.

(sexual) existence. It has been positively demonstrated that the various plasmodia undergo an alternation of generations and require two different hosts for their development, i.e., mosquito, man.

The asexual development, or schizogony, takes place in the blood of man, the sporogony, or sexual development, in the body of the anopheles mosquitoes, the bite of which sets up an infection in man, since the sporozoites of the various plasmodia are developed in the salivary glands of these mosquitoes. In the act of biting, the sporozoites reach the erythrocytes where they become the intra-corpuseular hyaline bodies beginning again their asexual cycle of development in the blood.

That the mosquito is the intermediate host of the malarial parasite and that the infection in man follows bites by infected mosquitoes has been abundantly proven. The mosquitoes that act in this way are the various *anopheles*; the *Anopheles Maculipennis* being the offender most frequently. The freshly formed schizonts in the blood of an infected man are conveyed into the intestines of the mosquito. Here sexual reproduction of the parasite begins. The male elements, filamentous *microgametes*, penetrate the female elements, *macrogametes* (spheres), and after a time become mobile fusiform bodies, *ookinets*. These bore into the intestinal walls of the mosquito and there remain. After a time they are converted into round bodies, or *oöcysts*. The nucleus of the oöcysts divides rapidly and other daughter nuclei are formed, and new cells called *sporoblasts*. After about eight days these form the sporozoites. The number of sporozoites in each oöcyst varies from hundreds to many thousands (often 10,000). These oöcysts burst and the sporozoites in the circulation find their way to the salivary glands of the mosquito. When a mosquito bites a human being they are introduced into the blood where they are quickly transformed into the intracellular hyaline bodies and begin their *asexual sporogony* in the blood. Each developmental cycle causing a febrile paroxysm either every day or alternate days, or on every fourth day, etc., depending on the character of the organisms and the number of infections. To pre-

vent spread of malaria, mosquitoes must be prevented from reaching individuals infected with malaria and those not infected. Screens accomplish this best. The larva of the mosquito develops in stag-

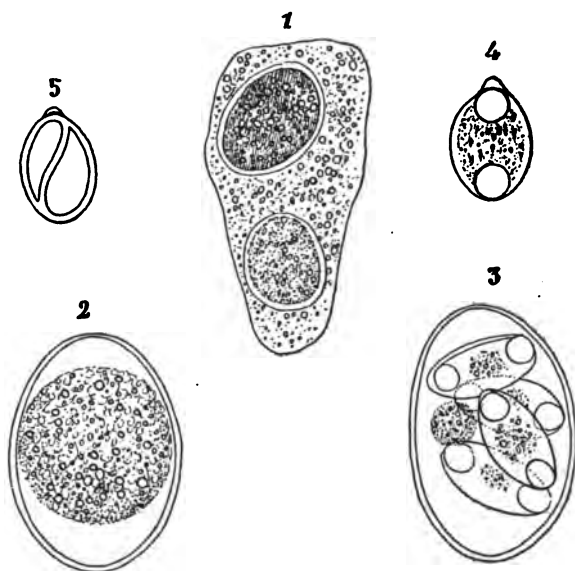


FIG. 61.—*Coccidium hominis*, from intestine of rabbit: 1, a degenerate epithelial cell containing two coccidia; 2, free coccidium from intestinal contents; 3, coccidium with four spores and residual substance; 4, an isolated spore; 5, spore showing the two falciform bodies— $\times 1140$. (From Railliet, in Tyson's Practice.)

nant water. To prevent the development of these young mosquitoes oil should be poured on the water, thus cutting off the air and means of respiration.

COCCIDIUM.

Coccidium hominis is another member of the sporozoa that occasionally infects man. Coccidia are infectious also for horses, goats,

oxen, sheep, pigs, guinea pigs, weasels and rabbits. The organism is essentially a cell parasite inhabiting the cells of the gastro-intestinal tract by preference, chiefly the liver and intestinal mucous membranes. They lead a sexual and asexual existence like the malarial parasites (alternate generation). The young sickle-shaped nucleated sporozoite penetrates an epithelial cell, where it gradually develops,

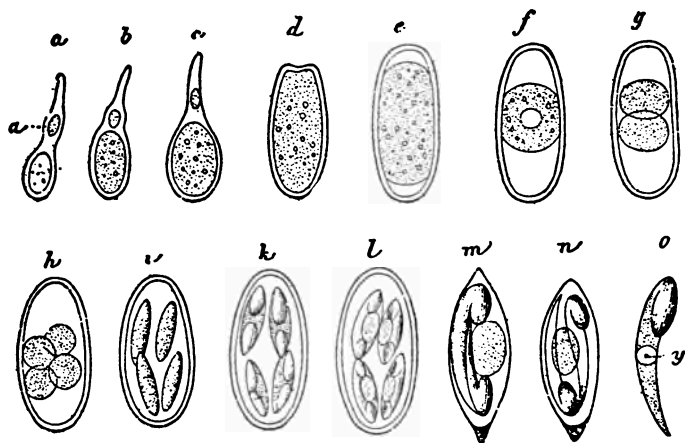


FIG. 62.—Development of *coccidium cuniculi*: *a, b, c*, young coccidia in epithelial cells of gall duct; *d, e, f*, fully grown encysted coccidia; *g, h, i, k, l*, showing development of spores; *m*, isolated spore, greatly magnified, showing the two falciform bodies (*pseudonavicella*; *sporozoites*) in natural position; *n*, a spore compressed so as to separate the two sporozoites; *o*, a sporozoite or falciform body with *y*, its nucleus. (From Railliet after Balbiani—in Tyson's Practice.)

ultimately dividing into numerous sporozoites. This is the asexual stage of development (schizogony), the sexual stage being called sporogony.

The sporozoites are differentiated into the two sex elements. These are large granular appearing cells, the male being smaller, divides into numerous flagellated microgametes that penetrate the female granular cell, macrogametes, and fertilize them. These microgametes form capsules and become oöcysts which divide into

numerous sporoblasts, changing into sickle-shaped sporozoites upon liberation.

The coccidia are easily demonstrable in tissue and in feces. They produce in man occasionally a fatal disease infecting the liver and intestines. Cattle sometimes die from hæmorrhagic dysentery due to one of the coccidia. The disease is transmitted by the ingestion of food contaminated by feces containing the sporozoites.

Acid fuchsin stains the sporozoa.

PYROPLASMA HUMANIS.

A protozoon supposed to be the cause of spotted fever in the valley of the Bitter Root river, Montana. This disease is a febrile one characterized by an irregular fever range, by muscular pains, arthritic involvement, petechia, and purpura in the skin. It is supposedly infectious, but not contagious. Its cause is considered by Wilson and Chowning to be the protozoon *Pyroplasma*, which occurs in the blood of infected individuals. It appears within the erythrocytes and they resemble hyaline bodies of malaria. They are from 1μ to 2μ in length, sometimes from four to sixteen bodies are found within a single cell. They grow gradually larger and then exhibit amœboid motion with pseudopodia formation.

By injecting blood from an infected man into rabbits, the latter become infected, and the parasites are found in the blood. It is believed by the discoverer that the parasites are conveyed from the gopher *Spermophilus Columbianus* to man by the means of ticks.

DISEASES SUPPOSEDLY DUE TO ANIMAL PARASITES.

Hydrophobia.—This disease has long been considered to be an infectious one, but the causal parasitic agent has never been discovered. It is commonly found in dogs, cats, wolves, rabbits, etc., but other domestic animals, and man may become infected. It is a disease of the central nervous system, highly infectious, always

following a bite or other injury in which the skin is broken, and in which lesion the virus may be deposited. Infection may be caused in man by injecting emulsified infected nerve tissue (brain) into susceptible animals (rabbits or monkeys). The disease is always fatal after it is well established. Well-marked histological lesions of the central nerve tissues, particularly the large ganglia, have been found by Van Gehuchten and Nelis, and Ravenel and McCarthy. If emulsified brain tissue from an animal that has died of hydrophobia is ~~filtered~~ filtered through a "germ-proof" filter the filtrate is capable of setting up the disease in a healthy animal if it is injected into it. By long centrifugation of emulsified infected brain tissue, the supernatant fluid loses its power of reproducing the disease on injection.

The organism is toxic in character, since filtrates sometimes fail to produce transmissible disease, but emaciation, paralysis, and death are caused by their injection into rabbits, the tissues of which, in turn, are not infectious.

The unknown organisms are rather resistant to agents that are germicidal. They are destroyed in fifty minutes by a 5 percent carbolic solution, and in three hours by a 1-1,000 corrosive sublimate solution. Direct sunlight kills them quickly, so do radium emanations. The latter have been used as a curative measure with reputed success. A temperature from 52°-58° C. destroys them, but they resist extreme cold of liquid air (-312°) for many weeks. Pasteur found that desiccation attenuated the virus. Chlorine kills it quickly, while glycerine does not. The virus may be increased in virulence by passing the "street virus" of dogs through a series of rabbits. Here the period of incubation decreases from three weeks to six days, but beyond this the period does not become less, and the degree of virulence from the virus lead Pasteur to name it *virus fixe* (fixed virus).

Passing the virus through foxes, cats, and wolves also intensifies the virulence, while monkeys and chickens attenuate it.

Negri bodies, protozoon bodies discovered by Negri, are found in

the ganglionic cells of rabid animals. These bodies stain by eosin, and are from one to twenty-seven microns in size, being generally about five microns. They are found particularly in the cornu of Ammonis; in Purkinje's cells in the cerebellum; and in the larger cells of the cortex of the cerebrum. These may be the cause of the disease, but there are several objections to this hypothesis. Their distribution does not correspond to the parts of the nervous system that are most intensely affected in hydrophobia, i.e., medulla and pons. In the latter locality these bodies are rarely encountered. They are not found invariably in animals dead from rabies, and are considered to be too large to pass through a Berkefeld filter; this latter view may not be a correct one. The finding of these bodies has been considered by Negri to be good grounds for considering the case to be hydrophobia. The rapid diagnosis of the disease in animals can only be effected by killing them and examining the nervous tissues, or inoculating other animals with them. Histologically, three marked changes may be noted: 1. The finding of the Negri bodies. 2. The finding of the degeneration of the cells of the larger ganglia with the proliferation of the endothelial cells lining the ganglionic spaces (Van Gehuchten and Nelis). 3. The finding of certain tubercles in the medulla, which are called Babes tubercles, though these are not wholly characteristic, as they are found in other diseases. Hydrophobia is transmitted from the site of the wound to the central nervous tissues by the nerves, and the incubation period varies with the distance of the wound from the central nervous system.

Immunity against infection and the development of the disease after the reception of an infected wound, may be accomplished by Pasteur's method. (See chapter on vaccine.)

Yellow Fever.

That this disease is caused by a parasite there can be no doubt. It is highly infectious and largely confined to the tropical regions of the western hemisphere and in parts of Africa.

Like several of the protozoon parasites, this one is unquestionably spread by mosquitoes, and it has been definitely determined by Carrol and Reed that the ***Stegomyia Fasciata*** is the means of its propagation. Carrol believes that the undiscovered parasite of yellow fever is of the animal kingdom, for the following reasons:—

1. It is absolutely necessary for its continued existence that it undergoes *alternate generation* in man and in the *Stegomyia* mosquito. This is peculiar to the sporozoa.
2. The fact that two weeks must elapse before the mosquito is capable of infecting man is evidence that a cycle of development of the unknown parasite is taking place in the mosquito.
3. The limitation of the cycle of development of the parasites to a single genus of the mosquito and to a single vertebrate (man) conforms to a natural zoologic law, and this does not conform to our knowledge of the life history of bacteria.
4. The effects of climate and temperature on the life history of the *stegomyia*, and on the rate of development of the parasites in the bodies of the mosquitoes are exactly the same as the effects of the same conditions on the *anopheles* mosquito and the malarial parasite. Without the *stegomyia* there can be no yellow fever. Infection requires the fulfilling of the following conditions:—
 1. By the bite of the mosquito providing the insect has fed on the blood of a yellow fever patient within the first three days of the fever.
 2. The disease is not transferred immediately, but a definite incubative period of more than eleven days must elapse before the mosquito can transfer the disease. After twelve days the mosquito has been found to be infected for at least fifty-seven days.
 3. Yellow fever cannot be carried by fomites.
 4. Yellow fever may be produced in a healthy man by the subcutaneous injection of blood from a yellow fever case (parasites in the blood).
 5. The serum of a yellow fever patient filtered through a very fine Berkefeld or porcelain filter is still capable of setting up the disease if injected, proving that the infection agent is submicroscopic.
 6. An attack of yellow fever produced by the bite of a mosquito confers immunity against subsequent infection.
 7. The period

of infection is usually three days but may be from two to six days. 8. A house or ship may be said to be infected with yellow fever only when there are present *stegomyia* capable of conveying the parasite of the disease. 9. The spread of yellow fever may be prevented by destroying the *stegomyia* and preventing egress and ingress of the insects from yellow fever patients to the non-immune. 10. No insect, other than the *stegomyia*, has been found to be concerned in the spread of yellow fever.

Yellow fever is a tropical or subtropical disease, because the *stegomyia* is confined to these regions, and the disease is found in low moist localities rather than those that are drier and higher, from the fact that the mosquito inhabits the former and not the latter. Yellow fever dies out after the first sharp frost, because the *stegomyia* are then either killed or undergo hibernation. Many conclusive experiments by Reed and Carrol, by Guiteras, and by the French Commission have proved that the *stegomyia* is beyond doubt the cause of the spread of the disease.

Small-pox and Vaccinia.—These two diseases must be considered to be but two clinical activities of one unknown specific micro-organism.

Certain protozoonoid bodies have been seen by numerous observers, notably by VanderLoeff, L. Peiffer, and Guasniere. The latter gave the name *Cytorictes vacciniæ s. variolæ*. In the deep layers of the epithelial cells of the pustules of vaccinia and small-pox, in the experimental lesions on the corneæ of rabbits, and in the protoplasm of the cells, these bodies are found. They are about the size of a micrococcus and exhibit amoeboid movements in hanging drop preparations. They are perfectly characteristic of the lesion produced in vaccinia and are not found in other diseased conditions.

The filtered virus produces no lesions on rabbits' cornea, showing that the virus is of such size as to be kept back by the porcelain.

In variola many different changes occur in the appearances of these cytorictes, suggesting developmental cycles.

In variola they are often intra-nuclear, while in vaccinia they are never found within the nuclei.

The cycle of development is suggestive of the development of many of the protozoa. Stages of development exhibiting fusiform amoeboid shapes can be seen, and pseudopodia can be detected in the process of developmental stages suggestive of gametocytes; the union of the gametes and the ultimate formation of the zygote can also be discerned.

After the tenth day these bodies cannot be very well discerned in the tissues.

There is reason to think that the parasites circulate in the blood in variola. The contagion in variola is thought to be by inhalation. It is certain that the disease can be produced by inoculation with virus from a case of small-pox. The contagion exists in the scales, pus cells, and excretions of patients ill with small-pox.

If the virus of small-pox is introduced into a monkey and then into a cow the disease produced is not variola, but vaccinia (Monkman). The hypothetical organism above described, *cytocytes*, becomes attenuated in the cow, so that it is incapable of producing variola, but vaccinia.

Rabbits, horses, and sheep are susceptible of inoculation with the virus of vaccinia (see vaccination). Virus may be tested by rubbing over the shaven bellies of rabbits, setting up minute vesicles and finally crusts. (Calmette.)

Scarlet Fever.

Mallory in 1903, found certain bodies in the skin of scarlet fever cases. These bodies, he assumed, were protozoan in character and were the etiological cause of the disease. He named them **Cyclaster Scarletinas**. They have been found rather constantly in the skin of scarlet fever cases, also in the skin in cases of measles and in anti-toxin rashes.

By several observers they have been considered to be artifacts or degeneration products in the epithelial cells.

It must be said of both the hypothetical organisms of variola and scarlatina, that if they are the cause of these two diseases they differ from all other known protozoan parasites, because the latter require an intermediate host for the transmission of the parasite from individual to individual while these certainly do not.

FILARIA.

The condition known as filariasis in man is that in which round or nematode worms (embryos) are found circulating in the blood.

There are several well known varieties, i.e.,

Filaria Nocturna.—In which the worms appear only during the night.

Filaria Diurna.—In which worms appear during the day and disappear at night.

Filaria Perstans.—In which the worms appear at all times.

Filaria Demarquau.—In which they are apparent both night and day.

In general the filaria embryos are characterized by a sheath, except the perstans. They are generally actively motile, are pointed at both ends, and are bisexual.

Filaria Nocturna.

Filaria sanguinis Hominis.

The developmental cycle of the *Filaria nocturna* depends upon an existence both in the body of the mosquito and in man. The adult worm (female) measuring 94 mm. in length has been found in the lymphatics of the trunk and extremities, where it was first seen by Bancroft. The young embryos are hatched into the lymph stream, ultimately reaching the blood. In the blood they are only found after sunset, and why this is the case is not known. It is of interest to note that the appearing time of the young filaria in the circulation corresponds with the swarming time of the mosquitoes.

They throng in the lymph channels causing chyluria, lymphangitis, and elephantiasis. The embryo filaria is about 3 mm. in length and very slender. It is actively motile, is sharply pointed at both ends, and has a sheath and a proboscis covered by a prepuce at the head. It has a coiling lashing movement with a slight tendency toward locomotion. The movements continue for several hours until the blood dries.

They are extremely numerous in the blood, often from 140,000 to 3,000,000 are to be found in an infected man. It is more than probable that filaria reaches the blood by the bite of the mosquito. It has been found that the young filaria bore through the intestinal wall to the thoracic muscles of the mosquitoes, where they may lie for some time, until finally they reach the mouth of the mosquito.

The disease filariasis, is very widespread, being common in India, China, Japan, and Australia, in the Southern part of the United States, Brazil, etc.

The parasites are to be found in the blood after six o'clock in the evening, and keep increasing in numbers until midnight. Ten to twelve may be found under one cover-glass at a time. If the patient sleeps in the day, and is awake at night, the parasites are found during the day.

It has been suggested that the nocturnal habits of the mosquito influence the time of the appearance of the parasite. If these organisms disappear while the patient is walking about, a few days' rest in bed will favor their return to the circulation.

Filaria Perstans.

This is found in the embryonic state in the blood of negroes in Africa.

It differs from the other filaria in man by its small size, the absence of a sheath, great locomotive powers, contractility, and in the embryos being thinner. It occurs in smaller numbers in the blood.

The intermediate host of this organism is supposed to be a suctorial insect of some kind. It may be a mosquito, or a tick.

Filaria Diurna.

This organism, discovered by Manson in Africa, differs from the *filaria nocturna*, in the fact that it appears only in the blood of its host in the day time. This indicates that it was set at liberty in the blood of its host during the day. The mangrove fly is thought to be its intermediate host. The morphological characteristics do not differ enough from the *nocturna* to warrant a detailed description. They are supposed to be embryos of the *Filaria Loa*. All of the *filaria* may be seen in fresh blood preparations with the naked eye, or in stained preparations after staining with methylene blue.

CHAPTER VII.

STUDY OF BACTERIA.

Bacteria are studied in the following various ways:—

1. Morphological characteristics, form, size, motility, presence of spores, granules, capsules, and flagella. Reaction of protoplasm to dyes and reagents.

2. Characteristics of growth in culture media; appearances of culture; chemical activities; production of acid, gases, toxins, colors, etc.; reactions to heat, disinfectants, light, etc.

3. Study of the action of bacteria on the tissues of man and animals, and of the toxins on the tissues and functions of the various organisms. The simplest way to study bacteria is to make a hanging drop of a fluid containing bacteria, and observing the organisms under a microscope. To do this, a cover-slip is used and a slide with a concavity ground in it. A drop of bacteria laden fluid is placed on the cover-glass, and after the edges have been smeared with vaseline, the cover-slip is inverted over the concavity in the slide, and the bacteria can then be examined with either the dry $\frac{1}{8}$ inch, or the $\frac{1}{12}$ oil immersion objective. If the preparation is kept warm for some time, various vital phenomena may be noted. Direct division, sporulation, motility, agglutination, and bacteriolysis can be studied by this means. Instead of using a fluid, a block of nutrient agar may be cemented to the cover-glass; after the bacteria have been planted on the agar, the various vital phenomena may be noted.

All minute bodies, whether they be bacteria, dust particles or granules of india ink in suspension, exhibit a trembling vibrating motion called the Brownian motion. Motile bacteria either move so swiftly that the eye can hardly follow them, or they may merely

roll or waddle across the field slowly. Direct division and sporulation occupy several hours, and to observe these actions the preparation must be kept warm by putting the microscope in a warm box.

The Gruber-Widal reaction is thus studied. A drop of the serum and bouillon culture, mixed in proper proportions, is dropped on a cover-slip, which is then placed, drop downwards, over the cavity of the slide (*hanging drop* fig. 63).

Staining bacteria is a matter that is easily accomplished, and very many staining solutions and methods have been invented for this purpose.

The simplest procedure is to take a drop of pus, blood or culture, and spread it upon a very clean slide with a sterilized platinum needle. The matter must be spread thinly and evenly. After the water has evaporated and the preparation has become dry, it must be fixed.



FIG. 63.—Hanging drop, over hollow ground slide. (Williams.)

To do this various agents are used. The object of the fixing is to coagulate the protoplasm of the cells, and to fasten all the smeared matter fast to the glass, so that the staining fluid and water will not wash them off. This is accomplished, in the case of a slide, by holding it in the apex of a bunsen flame for about ten seconds. If a cover-slip is used it must be passed through the flame three times rapidly. After fixing, the staining fluid is poured on, and after remaining a few minutes is poured off and the slide is washed, dried by blotting paper, and examined. If a cover-slip has been used a drop of balsam is put upon a clean slide and the cover, smeared with stained bacteria, is inverted on the balsam. Upon the stained bacteria themselves (if a cover-glass has not been used) or upon the cover-slip a drop of cedar oil may be placed, and the preparation examined with a one-twelfth objective. This is one of the simplest

staining procedures practised in bacteriology. Other more complicated methods will now be described.

Besides heat, absolute alcohol, methyl alcohol, or formalin may be used as fixatives. Some stains are made up with methyl alcohol, and instead of fixing by heat, the stain is merely dropped upon the dried film, and the bacteria are fixed and stained by the same solution at the same time.

Aniline dyes are almost entirely used as stains in bacteriology and these are divided into two classes, the basic and acid stains, according as their staining properties depend upon the basic, or acid part of the molecule. Basic dyes stain nuclear tissues of cells and bacteria. The acid are used as contrast stains and do not color bacteria, but tissues in which they may be imbedded.

The common basic stains are methyl violet, and gentian violet, methyl green, methyl blue, and methylene blue, thionin blue, Bismarck brown, fuchsin, and saffranin. These are used for staining different bacteria under different conditions. The most useful stain is methylene blue, since it is difficult to overstain with it, and is very easily applied. It has been found that certain physical and chemical conditions are necessary for successful staining with aniline dyes. Alcoholic solution of dyes entirely devoid of water do not stain, absolute alcohol does not decolorize bacteria after staining with aniline colors, while diluted alcohol decolorizes readily. The more completely a dye is dissolved, the weaker is its staining power. A dye stuff unites, as a whole, with the bacterioplasm, forming, as it were, a double salt between the two. Certain substances, alkalies, carbolic acid, iron and copper sulphate, tannic acid, alum, and aniline oil, are added to a solution of aniline dyes, and they act as mordants, or fixatives, making the dye bite into the protoplasm of the bacterial cells. Spores, capsules, and flagella, are hard to stain, and special heavily mordanted stains are used to demonstrate them. Chemical reaction occurring in the cell protoplasm is of great value in differentiating bacteria. The presence of granules in bacterial cells is often only shown by the use of special stains, which deeply

color them. Bacteria of the tubercle group are called "acid fast," because, after being stained, it is difficult to decolorize them with acid solutions. These bacteria are hard to stain and resist decolorizing agents after they are stained.

1. Löffler's alkaline methylene blue solution consists of

Saturated alcoholic solution of methylene blue..... 30 cc.
 $\frac{1}{10000}$ solution caustic soda solution in water100 cc.
 Mix.

This is the most useful of all the staining mixtures employed.

2. Zeihl's solution carbol-fuchsin consists of

Fuchsin.....1 gram.
 Carbolic acid crystals.....5 "

Dissolved in 100 cc. of water, to which is added 10 cc. of absolute alcohol.

This can also be made by taking a 5 percent solution of carbolic acid in water and adding sufficient saturated solution of fuchsin in water until a bronze scum persists upon the top. This is used for staining tubercle bacilli in sputum and sections. It must be heated when used.

3. Fuchsin solution.

Saturated alcoholic solution of fuchsin..... 1 cc.
 Water.....100 cc.

4. Bismarck brown solution.

Water.....100 C.C
 Bismarck brown sufficient to saturate.
 Filter and use as contrast stain.

5. Weigert's aniline gentian violet stain.

Gentian violet 1 gram.

Dissolve in absolute alcohol 15 cc.

Distilled water..... 80 cc.

Then add to this

Aniline oil..... 3 cc.

Mix, shake and filter.

This stain can also be prepared by taking a

Sat. watery solution of aniline oil..... 100 cc.

Filter, then add

Sat. alcoholic solution gentian violet..... 10 cc.

This is a very intense bacterial stain used for demonstrating bacteria by the Gram method.

Gram's method of staining.

A cover-glass is spread with a smear of bacteria, or pus to be examined. After air-drying it, and fixing it in the flame, the aniline gentian violet is poured on, allowed to stand for a minute or two, then poured off and the preparation treated with

Iodine crystals..... 1 gram.

Potassium iodide..... 2 "

Water..... 300 cc.

for two minutes. This renders the purplish preparation grayish in appearance. Alcohol is now poured upon the preparation repeatedly until the alcohol does not dissolve any more color. A contrast stain of Bismarck brown is now used. If the bacteria on examination remain a dark violet blue they are then said to stain by Gram's method. If they are decolorized they are said not to stain by this method.

Many bacteria stain in this way, and many do not. Important bacteria often may be differentiated in this manner.

Thionin Blue, or Carbol Thionin.

This is a useful stain, prepared thus:—

Thionin blue	1 gram.
Carbolic acid	2.5 "
Water	100 cc.

Filter. Good for staining bacteria in tissues.

Special Stains.

Wright's Stain.—This not only stains, but fixes. It has a wide range of usefulness in a bacteriological laboratory for the staining of blood, pus, malarial parasites, trypanosomes, as well as many bacteria, and is prepared as follows:—

1% solution of sodium bicarbonate	100 cc.
Methylene blue	1 gram.

Mix and heat in sterilizer one hour at 100° C. Cool, then mix $\frac{1}{10}$ percent yellowish eosin in water until the mixture loses its blue color and becomes purplish. About 500 cc. of eosin solution are required. In mixing the two stains together a precipitate, eosinate of methylene blue, is obtained which is removed by filtration, then dried and added to 100 cc. of methylene alcohol until saturated. Filter after a few hours and add 25 cc. more of methyl alcohol so that the solution is not completely saturated.

This complex stain represents a type of which Jenner's, Leishman's, and Romanowsky's are members. To use this stain, a blood or pus film is spread and air dried. The stain is then run on the slip, or slide, for one minute. After this time slowly drop water on until the preparation becomes reddish. This is when the true staining takes place. After three minutes wash in distilled water, dry and mount. Nuclei, malarial parasites, trypanosomes, and bacteria are stained blue; red cells are stained pinkish-orange; while the granules of the leucocytes are stained pink, lilac, or blue, depending upon their character.

Giemsa's Stain.

This stain is used for demonstrating the newly discovered organism of syphilis—*Treponema Pallida* (*Spirochæte Pallida*) and is prepared as follows:—

Azur II Eosin.....	3 grams.
Azur II	8 "
Glycerine C. P.....	250 cc.
Methyl alcohol	250 cc.

1. Air dry the specimen.
2. Harden and fix in absolute alcohol.
3. Dilute stain with distilled water, using one drop of stain to each cubic centimeter of water.
4. Cover preparation with dilute stain 15 minutes.
5. Wash in running water.
6. Blot and mount.

Capsule Staining.

Bacteria are often covered with capsules that are difficult to stain, and special methods have been devised to demonstrate them.

Welch's Method.

1. Cover-glass preparations are made in the usual manner, and over the film, after fixing, glacial acetic acid is poured.
2. Without washing off the acid, aniline water gentian violet is poured on. Change the stain four or five times to remove the acid. Stain four minutes. This demonstrates the capsule very well.

His's Method.

"A".

1. Make cover-glass preparation as usual. Fix in flame.
2. Stain for a few seconds with a half concentrated water solution of gentian violet.
3. Wash in weak potassium carbonate solution for a few minutes.

4. Dry and mount.
- "B".

1. Dry and fix.
2. Heat and pour on the following stain:
 - a. Saturated alcoholic solution of gentian violet.... 5 cc.
 - b. Water95 cc.
3. Wash in a 20 percent solution cupric sulphate.
4. Dry and mount.

Spore Staining.

Spores resist stains, and when stained are hard to decolorize.

1. Dry and fix in the usual way.
2. Flood cover-glass with hot carbol-fuchsin; heat until it steams; repeat this once or twice. This stains bacteria and spores.
3. Wash in water.
4. Decolorize with

Alcohol	2 parts.
1% acetic acid.....	1 part.
5. Wash and dry.
6. Dry with methylene blue.
7. Wash, dry and mount.

By this method, which is a simple and satisfactory one, the spores are stained a brilliant red, while the body of the bacilli are stained blue.

Flagella Staining.

To a beginner flagella staining is difficult; there have been many well known methods devised. The simpler are as effective as the more complicated but do not always make as pretty preparations.

Flagella, being processes extending from the capsule, are, like the latter, hard to demonstrate. They are not stained by the common bacterial stains. In general a powerful stain mixed with a strong

mordant must be employed. Some methods appear to be not so much a staining method in the ordinary sense but either a precipitating of the stain in the substance of the flagella or else a decomposition of silver salts in the flagella substance. To stain flagella, a young culture grown on agar must be employed; glycerine agar must never be used. A mass of the organism is gently mixed with a drop of distilled water until a uniform emulsion is made. A dozen cover-slips carefully washed and cleaned by alcohol are thoroughly flamed in order to remove the slightest trace of grease. The watery emulsion of bacteria is then spread over the cover-slips evenly and thinly. After they are dry the bacteria are fixed by holding them for a minute just above the apex of the flame with the fingers. The following methods may be pursued:

Pitfield's Method Modified by Muir.

Two solutions are necessary for this method.

A. Mordant.

10 % watery solution tannic acid	10 cc.
Corrosive sublimate saturated water solution	5 cc.
Carbol-fuchsin solution	5 cc.

This forms a dense precipitate which must be removed by the centrifuge, or sedimentation, and the clear fluid, or mordant, is stored in a bottle. It keeps for two weeks.

B. Stain.

Saturated watery solution of alum	10 cc.
Saturated alcoholic solution gentian violet	2 cc.

This keeps but two or three days.

The method of procedure is to flood the cover-slip with the mordant and gently steam for one minute, then wash and dry thoroughly, pour the stain on and steam for one minute more. Wash, dry and mount.

This method yields very good results.

Pitfield's Method.

This is the simplest stain and the easiest to use, but does not give the good results that the previous one does. But one solution is needed, this is made in two parts and mixed.

- A. Tannic acid 1 gram
Water 10 cc.
- B. Saturated watery solution alum (old) 10 cc.
Saturated alcoholic solution gentian violet 1 cc.
Mix.

A heavy precipitate is formed by this process which is useful in the staining. The stain is almost a saturated solution of alum and tannic acid, and when it becomes supersaturated by evaporation and heat, staining takes place. After this the process is very simple. The cover-slip is carefully flooded with the stain and warmed for a minute over the flame of a bunsen burner, turned very low, until steam arises. Not too much stain should be run over the cover-slip. After steaming occurs, the stain should remain for a minute, then the preparation is washed, dried, and mounted. It will be found that the best stained flagella are on those bacteria nearest to the edges where the evaporation has been most intense. If the preparation is not equally stained, Weigert's aniline gentian violet can be run on for a minute to deepen the color.

Löffler's Method.

This is the original flagella stain and is a very good one. It is made as follows:—

- A. Mordant
20% watery solution tannic acid 10 cc.
Sat. solution ferrous 5 cc.
Fuchsin sat. alcoholic solution 1 cc.
Mix.

- B. Stain
Carbol-fuchsin.

Proceed as in the previous methods.

The most important steps in flagella staining are to clean the cover-slips thoroughly, to mix the culture with water and have no culture media with it, to fix gently, and to not overheat the stain. Even in expert practised hands it is not always easy to demonstrate flagella readily.



FIG. 64.—B. Diphtheria stained by Neisser's method. (Williams.)

Neisser's method of staining the diphtheria bacillus.

Two stains are needed: (Fig. 64.)

- | | |
|---------------------------|----------|
| A. Methylene blue..... | 1 gram |
| 95% alcohol..... | 20 cc. |
| Water..... | 950 cc. |
| Mix and add | |
| Glacial acetic acid | 50 cc. |
| B. Vesuvium..... | 2 grams |
| Distilled water | 1000 cc. |

The staining steps are as follows:

1. Prepare film, fix and dry.
2. Pour on "A" for thirty seconds.

3. Wash well in water.
4. Dry and pour on "B" for thirty seconds.
5. Wash, dry and mount.

The protoplasm of the bacilli will be stained brown, and the characteristic (diagnostic) chromatin points will be stained a deep blue black.

Tubercle Bacillus Stain.

1. Spread the sputum, pus or culture, over the surface of the cover-slip. Allow the preparation to thoroughly dry.
2. Fix in flame and cool.
3. Pour carbol-fuchsin over the slide and either boil gently twice, or else heat for five minutes. Young bacilli in tubercles and other fluids are very difficult to stain in this way. The preparation containing them should be stood in cold carbol-fuchsin for twenty-four hours. This method stains everything on the slide.
4. Wash in water.
5. Decolorize the preparation with a 25 percent solution of sulphuric acid in water until the red color is lost. Repeat this once or twice.
6. Wash and counterstain with Löffler's methylene blue.
7. Dry and mount.

In such a preparation, if tubercle or other acid-fast bacilli are present, the bacilli will be colored a brilliant red, while the pus cells, epithelial cells, and other bacteria will be stained blue.

CHAPTER VII.

BACTERIOLOGICAL LABORATORY TECHNIC.

In order to study bacteria by other methods than the simple examination of their morphology by means of stains, and by the hanging drop, or block method, they must be cultivated either in the bodies of experiment animals, or else in culture media artificially prepared. The latter method is the most widely used in laboratories. It is necessary, in order to study bacteria, that the media shall not contain any extraneous bacteria to begin with, and that they shall be cultivated under such conditions that these bacteria cannot reach the media at any time. To accomplish all this, the culture media must be kept in glass vessels, such as test-tubes and flasks that have been sterilized. And, since all animal and vegetable substances, not actually alive, are overwhelmed with a multitude of bacteria, these substances must be sterilized too, in order that the media shall be free from any living organisms.

Glassware, such as pipettes, Petri dishes, flasks and test-tubes, are sterilized best by dry heat in hot air sterilizers. The apparatus is subjected to a temperature of 150° C. for one hour, or until the cotton plugs are slightly brown. The glassware should be put in wire baskets and the test-tubes should be kept erect. Petri dishes are best sterilized in a wrapping of paper. Flasks and test-tubes are always plugged with raw cotton, which prevents the ingress of bacteria, while air can reach the media through it freely.

Sterilization of culture media is accomplished in steam sterilizers of two patterns; of these, the autoclave, using steam under pressure, is the most satisfactory and is most generally used at present.

The baskets containing the culture media are placed in the auto-

clave after two quarts of water have been put in it. The lid is screwed down and the flame started; free flowing steam should escape from the valve before the latter is shut. When the pressure has risen to one atmosphere (15 pounds) or 120° C. for twenty minutes, all bacteria are destroyed, and the media can be safely assumed to be sterilized. If media containing sugar or gelatine is to be



FIG. 65.—Autoclave.

sterilized, the temperature should not run above 110° C., since, if this is done, the gelatine will not solidify when cold, and the sugar is caramelized and the media blackened.

Potato tubes are harder to sterilize at times, and it is safer to repeat the operation in twenty-four hours.

Fractional method of sterilization, or Tyndallization, is accom-

plished by heating the media to 100° C. on three successive days in a Koch or Arnold sterilizer. By heating culture media to this temperature, all the vegetative, or adult, forms are killed, while the spores are not affected; after the first sterilization, at room temperature, the spores vegetate and become adult bacteria, when on the second sterilization they are non-resistant to 100° C. and are killed.



FIG. 66.—Arnold sterilizer.

Any spores remaining after this develop into adult forms again and are killed on the third day, at the third sterilization. This fractional sterilization is employed in many laboratories still. To be effective, the media must be exposed to a temperature of 100° C. for thirty minutes, that is, thirty minutes after the steam has begun to form.

Bacteria that grows best at a temperature of 37° C. (most of the pathogenic ones do) develop more rapidly and luxuriantly in an incubator, or thermostat. Indeed some organisms, like the tubercle bacillus, cannot be cultivated without it. An incubator comprises an air chamber surrounded by a water chamber, and this, in turn, is surrounded by another air chamber. It is essential that the interior of the incubator be kept at an even, unvarying temperature;

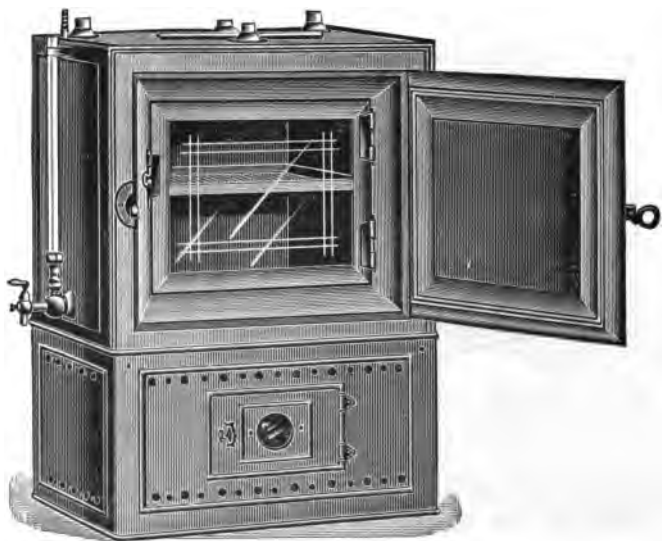


FIG. 67.—Incubator.

this is accomplished by using a small bunsen flame under the incubator. The heat from the flame warms the outer air chamber or jacket, and it in turn warms the water jacket, and the interior air chamber, where the cultures are kept, is thus heated to the required temperature. The amount of heat is automatically regulated by a thermo-regulator, which diminishes the gas supply if the temperature

runs too high, or increases it if it runs too low. The Roux regulator is the simplest and most efficient one.

A serum coagulating apparatus is needed in laboratories in order to coagulate the tubes of blood serum. (Fig. 68.)

Serum tubes are coagulated in it at a temperature of about 70°C . They are then sterilized by heating them for an hour at this temperature, for five successive days.

The separation of bacteria from the bouillon in which they grow



FIG. 68.—Blood serum coagulating apparatus.

for the preparation of toxins requires the use of a bacteria or germ proof filter, the best type of which is the Chamberland or Pasteur unglazed porcelain filter. These filters are of varying grades of fineness, and are so made as to be easily sterilized. The common pathogenic bacteria cannot pass through the pores of the ordinary filter, but toxic agents are known to pass through the finest filters, though they cannot be discovered, as they are submicroscopic.

To operate the porcelain filter it must fit into the neck of a vessel very tightly, so that a vacuum may be maintained about it by means of an air pump.

Collodion sacs are sometimes used in animal experiments. Bouillon

cultures are placed within the sacs, which are then inserted in the abdomen of an animal and left there. The sac is made of collodion because it is non-absorbent and allows the bacterial juices and products to osmose outwards and be absorbed by the animal, while the animal fluids percolate into the sac. There are several very ingenious ways of making these sacs, but the details are too elaborate to be described here.

BOUILLON.

Bouillon or broth is the most useful of all the nutrient media, since it is not only used as a liquid media, but by the addition of gelatine, or agar, it is converted into solid media.

There are two methods of making bouillon.

Method 1.

Take 500 grams of lean beef free from all fat, chop it fine and cover with 1,000 c.c. of water, shake and place on the ice over night. Then squeeze the fluid out of the meat by means of a cloth, and supply enough water to make a litre. Bring to a boil and add

10 grams of Witte's peptone.

5 " common salt.

Weigh the saucepan and contents and heat to 60° C. Supply the water lost by evaporation. Neutralize either by adding sufficient sodium hydrate, 10 percent solution, until red litmus paper is colored a faint blue, or else titrate 10 c.c. of the mixture with a decinormal solution of sodium hydrate, using phenolphthalein as an indicator, and after finding how much of a normal solution is required to neutralize 990 c.c. (1,000 c.c.—10 c.c. used for titration) this normal



FIG. 69.—Kitasato filter for filtering toxins. (Williams.)

solution is added. The mixture thus neutralized is then boiled for five minutes and the weight restored. After boiling, from .5 percent to 1.5 percent normal hydrochloric acid solution is added and the acidity thus produced is spoken of as $+.5$ percent or $+1.5$ percent as the case may be.

Upon boiling, the albumins are coagulated by heat, and the phosphates are thrown down. The acid re-dissolves the latter. The former must be removed by filtration. The filtrate is a clear straw-colored fluid of an acid reaction which should not become cloudy upon boiling. This is then run into flasks or test-tubes and sterilized.

The second method is much more convenient, and is prepared by adding 3 grams of Liebig's beef extract to a litre of water, and adding the peptone and salt, as in the previous method, and proceeding as before. To filter the bouillon, the filter paper must be folded many times, and the funnel must be carefully cleaned.

GELATINE.

To make **gelatine**, bouillon is made to which gelatine is added in order to render it solid. The following steps are taken:—

- a. Take a litre of water in a saucepan and add chopped beef or beef extract as in bouillon. After standing over night squeeze the beef and extract the juice.
- b. Add 1 percent peptone, 5 percent salt, 10 percent to 15 percent best gelatine and weigh.
- c. Heat until ingredients are all dissolved.
- d. Neutralize, gelatine is highly acid and requires much alkali.
- e. Boil five minutes and restore weight, boil till albumin coagulates.
- f. Cool to 60° C. and add an egg well beaten up in water.
- g. Boil slowly till all the egg is coagulated. This clears the media of fine particles that are not removed by filtration. Add 5 percent normal hydrochloric acid.

- h. Filter through absorbent cotton on a funnel previously wet with boiling water.
- i. Tube and sterilize in autoclave for fifteen minutes at 110° C. Litmus, or lacmoid, or neutral red may be added to the gelatine as an indicator.

AGAR-AGAR.

To make agar:

- a. Take 20 grams of powdered or chopped agar.
- b. Add to 500 c.c. of water, place in a can in autoclave and heat to 120° C. Then cool.
- c. Add this to 500 c.c. of bouillon of double strength, making 1,000 c.c.
- d. Neutralize.
- e. Cool to 60° C.
- f. Add an egg to the mixture, stir.
- g. Boil till egg is coagulated thoroughly.
- h. Add 5 percent normal hydrochloric acid, and while boiling hot, filter through absorbent cotton wet with boiling water.
- i. Run into tubes. Sterilize. Slope the tubes for twelve hours and store in dark place.

To make glycerine agar add 6 percent of glycerine to the agar before neutralizing. To make agar for tubercle bacilli, veal bouillon must be employed, and glycerine must be added.

Litmus Milk.

Carefully skimmed milk, to which litmus has been added, is run into tubes and sterilized. This is a valuable culture medium. It is also a reagent.

Potato Tubes.

1. Wash some large potatoes and with a Ravenel potato cutter,

cut out semi-cylinders of potato. Immerse in running water over night, in order to prevent them from turning black.

Thrust absorbent cotton to the bottom of the tube and wet with distilled water; place the potato upon the cotton, then plug the tube and sterilize in autoclave twice. The tubes should be sealed.

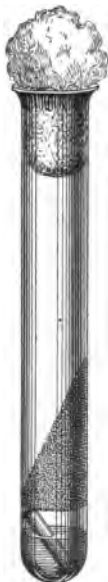


FIG. 70.—Potato in culture tube. (Williams.)

PEPTONE SOLUTION—Dunham.

Take Peptone.....	10 grams
Salt.....	5 "
Water.....	1,000 cc.

Mix. Boil. Filter and store in tubes and sterilize.

Dextrose and lactose culture media are often used. They are prepared by adding 1 percent of these sugars to the various media before neutralization.

BLOOD AGAR.

Is prepared by adding to agar some defibrinated rabbits blood, in varying proportions before the agar is tubed and hardened.

BLOOD SERUM.

The blood of a dog drawn under strictly aseptic precautions from a vein of an anesthetized dog is collected in a sterile jar and after the serum has separated, it is run into tubes by sterile pipettes and simply coagulated by heat. Sterilization is not necessary, and is harmful for the growth of the tubercle bacilli, because salts are formed which interfere with the growth of the bacteria.

LÖFFLER'S BLOOD SERUM MIXTURE.

Blood serum of an ox or a horse is employed, mixed with bouillon containing 1 percent of grape sugar.

Seventy-five percent of blood serum is mixed with 25 percent bouillon. This is run into sterilized tubes and the latter are placed in a blood serum coagulator and coagulated in a sloping position at a temperature of 65° C. or thereabouts.

After they are coagulated they are sterilized by heating an hour each day at 65° C. five successive days, or at 95° C. for an hour on three successive days.

After sterilization the tubes should be sealed carefully. Eggs are employed as culture media. The yolks and whites of a number of eggs are shaken together in a flask and then strained through a towel to remove the froth. The mixture is then run into tubes and coagulated and sterilized like blood serum. On this mixture the tubercle bacillus grows very well.

These are the common culture media used in laboratories. For a more technical description of the manufacture of these and other media, the student is referred to books devoted to laboratory technic.

The Study of the Growth of Bacteria.—Cultures.

Bacteria growing in colonies on culture media are spoken of as colonies. Aerobic bacteria may be made to grow on culture media by simply inoculating the media with some pus or blood containing them, by means of a sterile pipette or platinum needle. Bouillon may be thus inoculated, as may any of the media, and other cultures may be made from these by sterilized needles. But such cultures are made up of colonies of different sorts of bacteria—some pathogenic, some non-pathogenic, etc. To separate the various bacteria so that they will grow in isolated groups, is a comparatively easy matter, and is accomplished in several ways. The simplest is to employ several tubes of agar or blood serum. Over the surface of one of these, a platinum loop containing pus, or other matter, is rubbed successively. These tubes are then incubated. After a few hours, the first one exhibits a copious growth of many different

kinds of bacteria growing confluent together, from which it is impossible to isolate any pure cultures. The second tube is less covered with bacteria, while the third, instead of containing a mass of bacteria, exhibits tiny little dots, or colonies (pure cultures) growing discretely isolated. By means of a sterilized platinum needle these little colonies may be fished out and transplanted to fresh culture tubes, and after a few hours' growth they become pure cultures.

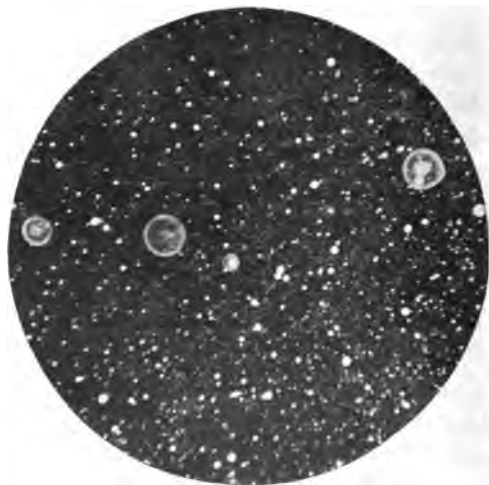


FIG. 71.—Colonies in gelatine plate showing how they may be separated and the organisms isolated. (Williams.)

An old method employed in many laboratories, in breweries and originated by Pasteur was what is known as the dilution method. Numerous flasks are inoculated by matter containing bacteria very highly diluted in bouillon or sterilized and by means of a sterile pipette drops of this highly attenuated mixture are dropped into flasks of sterilized bouillon or wort. Among a great number of flasks so inoculated, some will be found sterile, others will contain

two or three different forms of bacteria, while a few will, perhaps, contain a pure colony of the kind of bacteria for which a search is being made.

Another method is to inject some matter containing pathogenic

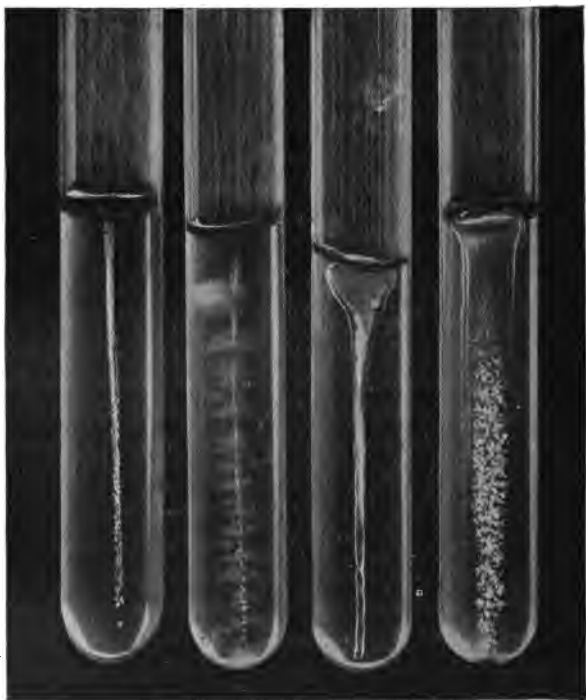


FIG. 72.—Series of stab cultures in gelatine, showing modes of growth of different species of bacteria. (Abbott.)

bacteria into a rabbit or guinea pig. The various juices and the leucocytes of the animal destroy the non-pathogenic bacteria and a pure culture, often of a pathogenic form, may be isolated from the blood or miliary abscess or tubercle of the animal at autopsy and

transferred to culture media. By far the most useful and ingenious method of procedure is the Koch, or plate method. This is used in many laboratories all over the world.

Koch was the first to employ solid culture media for this purpose, and his method depends upon the principle that a liquid culture media may be inoculated with bacteria and then spread out on sterile glass plates or dishes where it quickly hardens, the bacteria being uniformly separated from each other, and for a time at least kept isolated by means of the solid media, and after they have developed into isolated colonies they may be transplanted to tubes of media in which they may be stored. In another way if a man wanted to secure a pure lot of seed of a single variety from a multitude of many kinds, it would perhaps be impossible to pick out by hand the seed wanted because of their fewness and smallness, but if he sowed them and waited until the plants developed they could then be identified and gathered (Abbott). Thus it is with plate cultures.

To isolate a pure culture of bacteria, say the bacillus pyocyaneus from pus, the following procedure is adopted in this method.

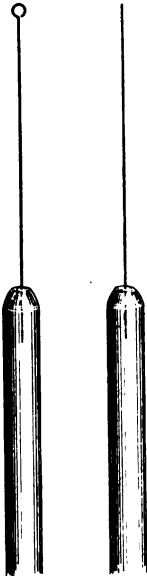


FIG. 73.—Needles used for inoculating media.

Three sterilized petri dishes, and three tubes of gelatine melted at 40° C. are used. A loopful of pus is taken up by a sterilized platinum loop and mixed with the gelatine of the first tube. To do this the tube is held across the left hand in a horizontal position and the cotton plug is removed, and held by its outside end between the fingers of the left hand, care being taken to prevent the tubal part of the plug touching anything and being contaminated. The platinum drop is then slowly and carefully introduced into the media, and stirred around so that the tube walls are not touched. The needle is again

sterilized and tube number two is held in the palm of the left hand parallel to the first one and its plug is removed also; then with a carefully sterilized needle, three loops of the inoculated gelatine are removed from number one and mixed with number two tube. The needle is then again carefully sterilized in the flame, the plug of number one is carefully replaced and another tube, number three, is held in the palm of the left hand and its plug is carefully removed and held as the previous ones were. With the sterilized loop three loopfuls of the gelatine from number two are



FIG. 74.—Method of inoculating culture media. (Williams.)

carefully introduced into number three and the needle is then sterilized and put aside. The petri dishes should now be laid on a cold level slab, and the contents of the tubes run into the different dishes. Tube number one is taken first; the lip of the tube is wiped with the cotton plug and then held in the flame to destroy all bacteria clinging to it. The lid of a petri dish is carefully and partially lifted and the contents of the tube rapidly and evenly poured over the bottom of the plate, and the lid quickly replaced.

This procedure is followed with the other tubes, and then the plates or dishes are put in a cool dark place, and the tubes are put into a solution of bichloride of mercury, or into boiling water.

The plates should be examined from time to time. After several days a perfect cloud of round colonies are seen in number one; a

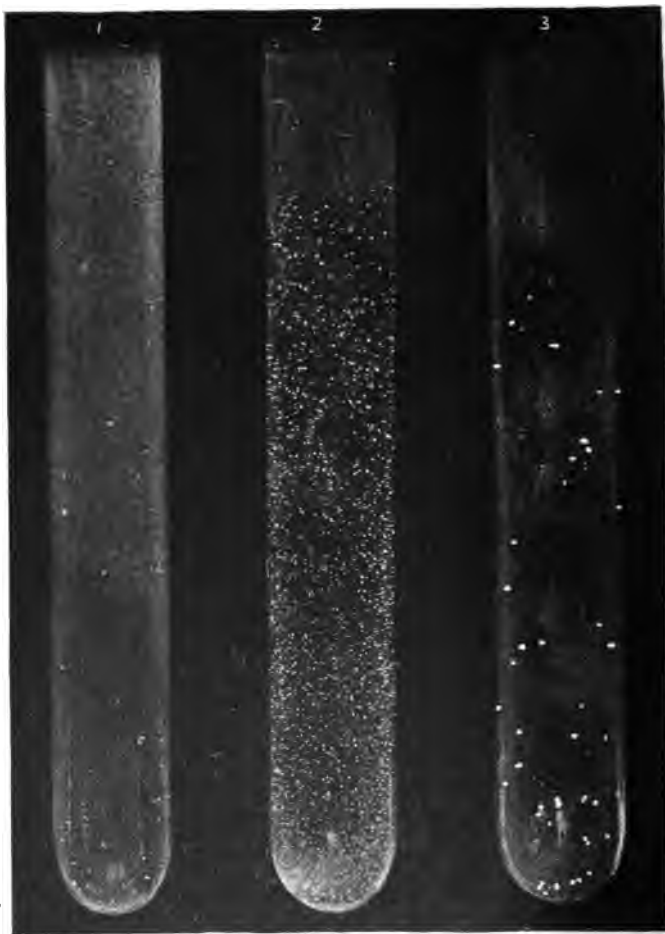


FIG. 75.—Dilution method of making cultures. 1, Is first tube containing great number of colonies; 2, contains less number; 3, relatively few. (Williams.)

large number in No. 2 and a much fewer number, say fifty, in No. 3. It is an easy matter then to pick out a colony that is surrounded by

a bluish green halo and transfer it to a tube of agar or bouillon. In the case of pus it is more than probable that the colony is that of the pyocyaneus bacillus, and that it contains nothing else but these bacilli. It must be studied in a dozen other ways, before it is certain that it is this bacillus, but the preceding method is a necessary primary step to secure this organism in pure culture and may be taken as a pattern for all plate methods.

Agar plates are often used since they have this advantage—they do not melt at incubator temperature. When agar is used it must be melted at 100° C. and cooled below 43° C. and above 39° C.

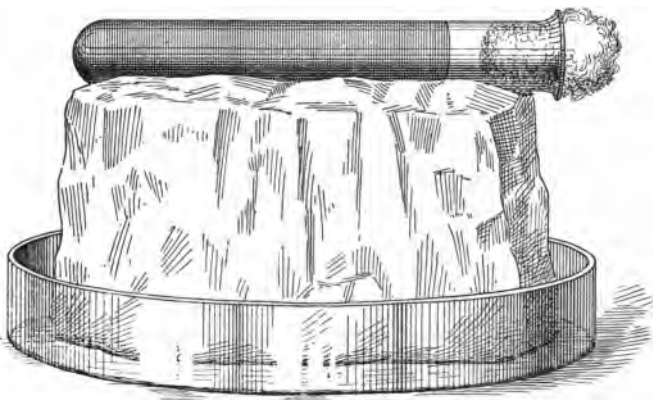


FIG. 76.—Esmarchs' method of making roll cultures on ice. (Williams.)

Above 43° C. bacteria may be killed. Below 39° C. the agar begins to harden, so this method must be performed quickly; the plates should be slightly warmed, the culture poured on and the agar hardened, they then must be inverted in the incubator, since the water of condensation forming in the lids of the plates often falls and washes one colony into another.

When gelatine plates are made, they must be kept in a cool place.

It is often of advantage to cool the plates, before they are filled, by means of ice.

Roll Culture.

Instead of pouring out the contents of the inoculated tubes the gelatine may be made to harden on the walls of the tubes by quickly rotating the tube in a groove melted in a block of ice. The centrifugal force distributes the gelatine over the glass, and the ice hardens it rapidly while in contact with the glass. Such tubes are veritable plates, and in them colonies of bacteria often grow as well as on the plates and may be fished out. The various characteristics of bacterial growth may be studied in cultures. Bacteria differ in very many ways in cultures. Some grow rapidly and luxuriantly; some discretely and slowly; colors and odors are produced by some; gelatine is liquefied by many, while others do not liquefy gelatine. Milk is curdled and digested by some; gas and acids produced by others. These various characteristics enable us to identify and differentiate bacteria.

The chemical activities of many bacteria are well displayed in milk culture. Milk is run into tubes, and sterilized tincture of litmus is often added to act as an indicator. Before using the milk, it must be skimmed and free from all fat.

The property of converting sugar into acids and gases is best studied in fermentation tubes.

Into sterile fermentation tubes bouillon containing sugar is run, these are plugged and sterilized. They may be inoculated with bacteria and if gas production occurs it is quickly manifested in the closed arm. The component gases may be studied and the various properties determined. This gas ratio is of use in identifying various bacteria and differentiating them. The closed arm of the tube being shut off from free air by the amount of bouillon in the open arm is practically an anaerobic tube and is employed for this purpose. Bacteria that grow in the closed arm are considered

anaerobes. By inoculating a gelatine tube with bacteria while it is melted and then letting it solidify, previously shaking the tube vigorously, gas formation will be speedily manifested by the presence of bubbles. Acids are detected in cultures by the employment of various indicators in the culture media. Litmus, lacmoid, and neutral red are used for this purpose. By titrating bouillon of



FIG. 77.—Fermentation tube. (Williams.)

previous known acidity with a decinormal soda solution, the amount of acid produced by different bacteria can be estimated.

Various sugars are fermented by bacteria, and lactic, acetic, and butyric acids are produced. Indol is also produced by many bacteria (colon bacillus, cholera bacillus), and its presence in culture is an important means of identifying different bacteria. The organism to be studied must be grown in culture media known to be free from indol. For this purpose, all meat extracts must be excluded and a simple solution of peptone and

salt, run into tubes and sterilized, is used. After bacteria have grown in this media for several days the indol produced, if it is produced, is detected by adding a few drops of pure sulphuric acid. If a red color (nitroso-indol) is not produced, a few drops of sodium nitrate solution (.02 grams to 100 c.c. of water) must be added, and if a pink to deep red color does appear it may be safely assumed that indol is present.

Ammonia is detected in culture by suspending a piece of paper wet with Nessler's reagent above a bouillon culture of a given organism. If a yellow to brown color is produced ammonia is present.

Nitrites are detected by growing the organism in a solution of a nitrate (see other works for description).

Incubate for a week and then add one cubic centimeter each of the following solutions:—

a. Sulphuric acid.....	.5 grams.
Acetic acid.....	150 cc.
b. Amido naphthaline.....	.1 gram.
Water.....	20 cc.
Boil, filter, and add 180 cc. of dilute acetic acid.	

If nitrites are present a pink color is produced by these reagents, enzymes may be detected by noting whether gelatine is liquefied, or milk curd digested. Both these actions are evidences of the presence of enzymes.

Bacteria growing exclusively in the absence of oxygen are known as anaerobes; to cultivate these successively various forms of apparatus are necessary.

The following methods are pursued in ordinary laboratory manipulations.

1. Exclusion of oxygen.
2. Exhaustion of oxygen by means of an air pump.
3. Absorption of oxygen by means of chemicals that absorb oxygen from the air. A mixture of pyrogalllic acid and sodium hydrate absorbs oxygen rapidly, leaving nitrogen only in the chamber.

4. Displacement of air by means of an air-pump and allowing hydrogen to enter the vacuum.

Under the *first* method we may either exclude oxygen by laying sheets of sterile mica or a cover-glass on the surface of the agar or gelatine plates (Fig. 78), thus excluding air, or deep punctures may be made in tubes half filled with gelatine or agar, for growths often occur in the depths of the media, especially if the latter has been boiled previously to



FIG. 78.—A streak made in agar by a needle inoculated with anaerobic bacilli and then covered at one spot with cover-glass. (Williams.)

expel the oxygen; or, instead of mica, sterile paraffine may be poured over the top of the tube. The layer of paraffine excludes the air. Flasks filled with bouillon, or tubes filled with bouillon, or melted agar may be inoculated with an anaerobic culture, but the filling of the vessel with the media must be absolute so that no space is left for air, otherwise the organisms may not grow. Roux employs a long sterile glass tube, which he completely fills with melted agar inoculated with the organism he wishes to grow. The ends of the

tube are then sealed in a bunsen flame and there being no air, anaerobic conditions are fulfilled, and organisms grow. After colonies appear the tube is broken at a file-mark near the colony and tubes inoculated therefrom. (Fig. 79.)

Under other methods large Novy jars are used for the reception of petri dishes and test-tubes. From these jars the air is withdrawn,

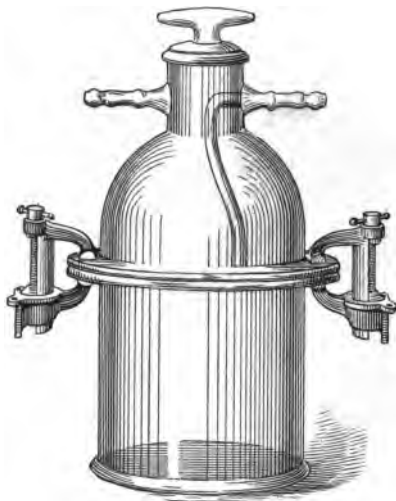


FIG. 79.—Novy jar.

and hydrogen allowed to flow into it. A solution of pyrogallic and sodium hydrate is placed in the bottom of the jar to absorb any remaining oxygen. There are many other ingenious mechanical ways of growing bacteria under anaerobic conditions and the student is referred to works devoted entirely to technic.

Animal Experiments.

To determine the pathogenicity of bacteria; to measure the strength of toxins and anti-toxins, to standardize anti-toxins, and to recover bacteria in pure culture, it is often imperative that small

laboratory animals be used. Guinea pigs, rabbits, and mice are oftenest employed. Strong young animals are the best. Culture toxins and pathological material are introduced into their bodies in various ways. A favorite one is to shave the abdomen, scour it with soap and water, and then bichloride of mercury, and finally sterile water. With a pair of sterile scissors a small hole is cut in the abdominal parietes and through it a loop containing a drop of culture is run into the peritoneal cavity, or under the skin. The animal is carefully weighed, and it is watched from day to day. If it dies an autopsy is made on it.

Other methods consist in injecting fluid culture into the veins of the ear, or into the peritoneum, by means of a sterile hypodermic syringe. The autopsy should be made carefully; the animal should be thoroughly wet with a solution of bichloride of mercury, then it should be stretched over a pan, especially devised for the purpose, or nailed to a board. The skin over the abdomen and thorax must then be shaved and sterilized with a solution of bichloride of mercury. The walls should then be seared in a line from the throat to the pubes with a hot knife, and through this line a cut should be made opening up the thoracic and abdominal cavities.

By means of a hot knife spots must be seared on the various organs, and with another sterile knife cuts should be made into the organs, then through these cuts sterile platinum needles are thrust, and then culture media is inoculated with them. Sometimes it is necessary to remove bits of tissue from various organs and place them in culture media. In the recovery of the tubercle bacillus from animals this procedure is necessary. Great care must be taken in making the culture and all tubes should be carefully stored. Often it is of great importance to make smears on cover-slips as well as cultures, from the heart cavities, liver, kidney, peritoneal cavity, etc., and stain them directly with Jenner's stain. It is sometimes necessary to inject cultures, or bits of nerve tissue from a rabies case into the brain. To do this, remove, under strict aseptic precautions, a button of bone from the skull by means of a trephine.

Histological Methods.

Sections of tissues from infected animals are often examined and stained by appropriate methods. To demonstrate bacteria, the tissues should be hardened in absolute alcohol, and imbedded in celloidin, then cut into sections and mounted in the following different ways.—

I. Löffler's Method.

- a. Float section in alcohol.
- b. Remove with section lifter to Löffler's methylene blue from five to thirty minutes.
- c. Decolorize in 1 percent solution of acetic acid for ten seconds.
- d. Dehydrate in absolute alcohol for a few minutes.
- e. Clear in xylol.
- f. Mount in balsam.

II. Weigert's Method.

- a. Transfer section to alcohol.
- b. Place in lithium carmine five minutes.
- c. Then in acid alcohol fifteen seconds.
- d. Wash in water.
- e. Transfer to slide and dry with blotting paper.
- f. Apply Ehrlich's gentian violet for three minutes.
- g. Blot and place in Gram's solution for two minutes.
- h. Wash and dehydrate in aniline oil.
- i. Wash with xylol.
- j. Dry, mount in balsam and examine.

In Löffler's method all the tissues, especially the nuclei and the bacteria, appear blue.

In Weigert's method, if the bacteria stain by Gram's method, the tissues appear pink, the bacteria a deep blue-black. This latter method is an admirable one. There are many other methods of staining. Paraffine embedding methods may be employed, but for these the student is referred to works solely devoted to technic.

CHAPTER VIII.

ANTISEPTICS AND DISINFECTANTS.

Many chemical substances have the power of entering into chemical union with the protoplasm of bacterial cells and so forming new compounds, and often coagulating the protoplasm.

Bacteria differ in their powers to resist these agencies; the anthrax spore is much more difficult to kill than the typhoid bacillus; these chemical substances act at a high rather than a low temperature.

A chemical disinfectant, such as copper sulphate, acts more rapidly and effectively in a watery solution than in a complex albuminous one.

It is often necessary to determine the exact minimum amount of an antiseptic that will destroy a given organism or produce a complete inhibition of growth; for this purpose small amounts of a disinfectant are added to gelatine in test-tubes and these are poured into plates and the result noted.

Previous to pouring the plates each tube is inoculated with a loopful of culture and thoroughly mixed with the media.

Another method is to make bouillon cultures of an organism and add to each a certain percentage of the solution of the antiseptic, and abstract every few minutes after the addition of the chemical one loopful of the mixture and inoculate fresh media.

It will be found in the case of most antiseptics in dilute solution that an interval of time must elapse before the organisms are killed. This is determined by observing the cultures made from the mixture. After five minutes, growth may occur, but after one hour, all may be dead, or it may take two or three hours.

The most valuable chemical disinfectants are those that kill in highly dilute solution in a short time.

Pieces of thread sterilized, and then put in fluid cultures may

be used in experiments; they are dipped into solutions of chemicals for varying lengths of time and then placed in culture media and growth noted.

Bichloride of mercury is a highly efficient germicide in watery solutions; if, however, albuminous matter is present its action is inhibited very much.

CHEMICAL DISINFECTANTS.

Mercury Salts.—Bichloride of mercury in highly dilute solution is a very valuable antiseptic. In 1-100 watery solution this salt will kill anthrax spores in twenty minutes. In blood, the anthrax bacillus is killed by a 1-2,000 solution in a few minutes. In bouillon the same organism is killed in a dilution of 1-40,000; in water, 1-500,000; all in the same interval of time. The presence of the albumins in the blood or bouillon, no doubt acts as a protecting envelope about the bodies of the bacteria. Bichloride is then more efficient outside the body than in it. It is also more useful and powerful when it is acidulated with a .5 percent of HCL, or when it is mixed with common salt or ammonium chloride. In culture 1-1,000,000 solution prevents the growth of most pathogenic bacteria. Biniodide of mercury is said by some observers to be more powerful than the bichloride. It is certainly less likely to be interfered with by albumins.

Sulphate of copper in water is a powerful germicide. It is more potent in watery solution than in bouillon. It has a remarkable affinity for algæ and for moulds. The author found that if moulds are put into alkaline solution of copper sulphate and heated, the copper enters into chemical union with the protoplasm of the mycelia, hyphæ, and the spores; 1-400,000 of copper sulphate in water destroys the typhoid bacilli. Even nascent copper kills the typhoid bacilli, so that copper foil in drinking water has the power, after a few hours contact, of destroying bacteria in the water.

The silver salts are useful in medicine as disinfectants, especially on mucous surfaces. The nitrate of silver is one of the most valu-

able of all the preparations, it is about a fourth as efficient as bichloride of mercury and is not nearly so toxic. Some of the albuminates of silver are useful because of their non-irritating action.

Acids, especially the mineral ones, are valuable disinfectants in not too dilute solutions. They act chiefly as inhibitors of growth rather than destroyers of bacterial cells. In the healthy stomach, hydrochloric acid acts as a normal disinfectant, and in disease, where it is absent, it must be added in order to prevent decomposition of food. Boric acid is useful in medicine on mucous membranes.

The halogens, iodine, bromine and chlorine, are active agents for the destruction of bacteria. The cheapest of these is chlorine. It acts best in contact with moisture, since it decomposes the molecule of water combining with the hydrogen to form free HCL and setting free oxygen.

Dry chlorine gas (45 percent) killed dry anthrax spores in one hour; when moisture was introduced 4 percent chlorine killed the spores.

Chloride of lime in 1 percent solution kills most bacteria in 1-5 minutes. Iodine preparations like chlorine ones are very powerful. They are of great use in medicine; ordinary tincture of iodine painted over infected areas acts as a powerful germicidal agent. It is too expensive to use in house disinfection and it is exceedingly destructive to all metallic objects. A 5 percent solution in 50 percent alcohol acts as a splendid disinfectant for intrauterine injection in puerperal sepsis.

Carbolic acid is valuable as a disinfectant because of its stability. A 1-1,000 solution inhibits bacterial growth; a 5 percent solution kills spores in a few hours. A thorough solution should be made, and to be very efficient, 5 percent HCL should be added to it.

Cresol, **lysol** and **creolin** are useful as disinfectants, but are sometimes unreliable since perfect solution cannot always be made. The mixture of one of these substances with water is more of an emulsion than solution. Anthrax spores have been known to live for hours in creolin solutions.

Peroxide of hydrogen has a great reputation in medicine as an antiseptic. It kills bacteria, especially the pus cocci, in a few minutes in a 15 percent solution. A 40 percent solution will kill anthrax spores in a few hours. It is a powerful agent when fresh, and is not poisonous. It combines with organic matter and becomes inert in time, however.

Formaldehyde gas, CH_2O , is, by all means, the most useful, as well as the most powerful disinfecting agent that we have. In solution 40 percent in water, it is known as formaline. It has a marked affinity for organic substances and forms chemical combinations with many organic bodies. When it unites with ammonia it becomes inert until some acid frees it. It unites with iron, but other metals are unaffected. Its use in medicine is wide and varied. It is deodorizer; renders gelatine, glass-like and insoluble in boiling water. It may be liberated as a gas in apartments and ships, actively destroying all bacteria. One percent of the vapor in the air of a closed room, if the air is moist, destroys bacteria after twelve hours. It is best to keep the room closed for twenty-four hours. It may be thrown into the room in many ways; by generators which decompose the vapor of wood alcohol, when they reach hot platinum sponges, salt, or hot copper; by vaporizing a solution by means of heat; by adding permanganate of potash to a solution of formaline; by spraying a concentrated solution over bedding, floors, and walls, then closing the apartment. It is very much more active in warm air than in cold, and when the air is moist. It has been known to destroy anthrax spores wrapped up in paper and placed under blankets. All of the pathogenic bacteria are killed by it, the staphylococcus aureus and anthrax spores being more resistant than anything else. It will not kill moulds unless highly concentrated. As dilute watery and alcoholic solutions decompose they should only be used when freshly made.

Sulphur Dioxide Gas.—An old and rather unreliable form of disinfectant. It does not kill anthrax spores very readily, as it requires an exposure of twenty-four hours to a 40 percent vapor

in a room. It is generated by burning sulphur in a room tightly closed, and it is much more efficient if water is vaporized in the room. It is not very penetrating, is poisonous to breathe, speedily bleaches fabrics, and attacks metal objects. It is much superior to formaline as an agent for the destruction of insects, especially mosquitoes.

Lime.—Ordinary thick lime, or whitewash, is highly germicidal. It is especially efficacious in disinfecting feces from typhoid cases. Typhoid bacilli are killed after one hour's exposure to a 20 percent mixture.

Potassium permanganate in 3 percent solution is said by Koch to kill anthrax spores in twenty-four hours. It is not so efficient a germicidal agent as supposed.

Turpentine and essential oils are efficient germicides in concentration. Common mustard rubbed in the hands is said to make them sterile.

Alcohol.—Ninety-five percent and absolute alcohols are not antiseptic for the anthrax spores, since they will live for many hours in contact with absolute alcohol. In general it is unreliable.

Zinc chloride in concentration is a powerful germicide. A 2 percent solution will kill the ordinary pyogenic bacteria in two hours.

Sputum, urine and dejecta are best disinfected by heat. Chemicals often are inert because they cannot penetrate the albuminous masses of the sputum or feces. Long contact with carbolic acid acidulated with HCL is very efficient. Concentrated formaline and solutions of chloride of lime may be used, also a heavy mush of lime in water.

Boiling or heating instruments and dressings by high moist heat, as in an autoclave, is the most reliable method of rendering them sterile. The exposure of dressings to 150° C. for one hour, or boiling instruments for twenty to thirty minutes makes them certainly sterile.

Disinfection of the skin is a difficult undertaking from a bacteriological standpoint. In the deep layers of the skin, and in the sweat glands and hair follicles, bacteria often exist, even after the most

thorough and prolonged disinfection. The application of soap and water with a stiff brush is by all means the most valuable part of the process, since with the removal of the dirt most of the bacteria are removed. Thorough scrubbing with soap and sterile water, followed by scrubbing with a 1-1,000 bichloride solution, cleansing the nails with a sterile brush, and prolonged immersion in bichloride or permanganate of potash solution, complete the process. Modern methods, even after all this preparation, require the use of rubber gloves that have been sterilized by boiling. The faultiest part of the preparation for an aseptic operation from a bacteriological standpoint, has always been considered to be the sterilization of the hands, and if these can be covered by rubber gloves that are sterile, the fault can be surely eliminated.

Antiseptic Values. (After Park.)

The figures refer to the relative antiseptic powers of various agents.

Alum	I to	222
Aluminium acetate	I "	6,000
Ammonium chloride.....	I "	9
Boric acid	I "	143
Calcium chloride	I "	25
Calcium hypochlorite	I "	1,000
Carbolic acid	I "	333
Chloral hydrate.....	I "	107
Copper sulphate.....	I "	2,000
Ferrous sulphate	I "	200
Formaldehyde 40%	I "	10,000
" pure	I "	20,000
Hydrogen peroxide, <i>fresh</i>	I "	14,300
Mercuric chloride	I "	40,000
" iodide.....	I "	25,000
Quinine sulphate.....	I "	800
Silver nitrate	I "	12,500
Zinc chloride	I "	500
" sulphate.....	I "	20

CHAPTER IX.

BACTERIOLOGY OF WATER, AIR, AND SOIL.

Bacteriological examination of water is of importance for the determination of the presence of pathogenic bacteria, and for the enumeration of the total number of all bacteria contained therein, the latter being considered an index of the purity of the water.

Several well known pathogenic bacteria have been found in water; among these are the typhoid, anthrax, cholera, plague, and colon bacilli, also the pus cocci. Since the tetanus bacillus is a normal inhabitant of the cultivated soil and manure, it is not at all uncommon to find it, at times, in muddy waters.

Bacteriological examinations of water are, in a measure, very disappointing, because it is very difficult, and at times impossible to determine the presence of the typhoid bacillus, even when it is certain that it is present.

The fact that the colon bacillus is always found in water contaminated by feces is a great help in the recognition of foul water. In the case of typhoid contamination the typhoid bacillus may elude detection, but the colon bacillus is easily found; we may then assume that, since it is impossible for typhoid bacilli to reach water without the colon bacilli that water having no colon bacilli is also free from typhoid bacilli. Also water having colon bacilli in great numbers is contaminated with feces, and perhaps typhoid feces. The detection of the colon bacillus is therefore of prime importance in the examination of drinking water. Its detection is simple. Water must be collected in sterile bottles, using every precaution against accidental contamination. Fermentation tubes are employed, containing bouillon with 1 percent of glucose. Into a series of these tubes, varying amounts of water are run by means of a sterile pipette, 2 cc.,

1 cc. .5 cc., .1 cc., .01 cc. of water being used. After a stay of twenty-four hours in the incubator, if gas appears, the bouillon should be examined by plate cultures for the colon bacillus. Lactose litmus agar is used, and where colonies appear that redden the litmus and resemble the colon colonies in appearance, they are planted in milk, fermentation tubes, peptone solution, neutral red agar, nitrate solution, and gelatine, and the various reactions in the various media noted.

The significance of the colon bacilli is often overestimated. They are found in all rivers, and often reach streams, wells, and even springs by contamination from the barnyard, or manured fields.

Typhoid bacilli have been found in water. One way that is sometimes successful is to take 25 c.c. of a 4 percent peptone solution and add this to a litre of the water to be examined; from this, after twenty-four hours in an incubator, plates may be prepared with the agar media of Drigalski and Conradi. This media is made of 3 percent agar, to which nutrose has been added and crystal violet. In the following manner add two litres of water to three pounds of beef, straining and boiling for an hour; after filtering, add twenty grams each of nutrose and peptone, and ten grams of salt. Sixty grams of agar are then added and the mixture boiled and filtered after being rendered alkaline. Boil 300 c.c. litmus solution with thirty grams of lactose, mix with the foregoing and alkalinize with a soda solution, and then add to this 4 c.c. of a 10 percent soda solution, and 20 c.c. 1-1,000 crystal violet (Höchst B.). Mix these solutions together, tube, and pour on plates, spreading the feces or water over the agar, dry and invert in the incubator twelve to twenty-four hours.

The typhoid colonies in this medium appear less granular and dark than do the colon colonies.

Typhoid colonies 1-3 mm. in size appear blue, colon colonies red, all other bacteria are temporarily inhibited by the crystal violet. Transfer the colonies to bouillon and test with a highly diluted serum from a horse artificially immunized, by the agglutination test.

To Count Bacteria in Water.

The sample must be collected in a sterile bottle, and the plates poured immediately, since bacteria multiply enormously after a few hours.

Take $\frac{1}{10}$ c.c. or $\frac{1}{2}$ c.c. or 1 c.c. of the water in sterile pipettes and mix with a tube of melted gelatine, pour quickly into cool sterile petri dishes and place in a cool dry place. After twenty-four to thirty-six hours count the colonies and the result (after multiplication where $\frac{1}{10}$ or $\frac{1}{2}$ c.c. of water was used) will be the number of bacteria per cubic centimeter. It may be necessary to dilute the water 5 or 10 times before pouring plates. A glass plate ruled into squares, known as a Wolffhügler plate, should be used for counting. The number of bacteria in potable waters vary in many ways, according to the amount of pollution, or albuminous matter in the water, while depth, and the swiftness with which it flows are conditions that modify bacterial contents. The water in a reservoir becomes free from bacteria during the first ten days. The number of bacteria diminishes 10 percent per day for the first five or eight days, due no doubt to gravitation of the bacteria to the bottom, also in part to the action of light, which plays an important rôle in the destruction of the bacteria of water supplies.

In general, water containing less than 100 bacteria per 1 c.c. is considered to be from a deep source, and uncontaminated by drainage. Deep artesian wells often contain but from 5 to 15 bacteria per c.c., water from rivers often contain 12,000 or 20,000, depending somewhat upon the season of the year. Rains cause an augmentation of the bacterial content. Summer causes a diminution.

In identifying a certain water supply as the cause of an epidemic of typhoid, the number of bacteria is of great value in locating the place of infection.

The efficiency of filters in large municipal water supplies is only known by the bacterial content of the effluent. In good sand and mechanical (alum) filters, the reduction in the number of bacteria

is often over 95 percent (sometimes 99 percent). Plate cultures should be made daily from every filter in order to determine how each filter is performing. Sand filters should not filter more than 1,000,000 gallons per acre a day. They should be at least one metre thick; the upper half inch of the sand performs over 90 percent of the filtration, due to a certain zooglea, or growth of bacteria. Cracks, or imperfections in the filter beds are quickly detected by the rapid increase of the number of the bacteria in the effluent. It is supposed that not only are bacteria filtered by the sand but that destructive changes occur in the filter which greatly diminish the number of bacteria. A filter must be used for a few days before it becomes efficient or "ripe." After a time it becomes inefficient and it must then be scraped, finally the sand must be removed and washed.

A sand filter is a highly efficient means of water purification. It often converts a foul dirty water into a bright, clean, wholesome water of low bacterial content.

Mechanical filters depend for their efficiency upon the addition of aluminum sulphate to the water. This is decomposed by the carbonates and aluminum hydroxide is produced, which is a white jelly-like flocculent precipitate, which mechanically entangles bacteria and removes them from the water. Mechanical filters, as a rule, are highly efficient. Domestic filters, even the Pasteur, are often unreliable.

In time of epidemics of cholera and typhoid even filtered water should be boiled before use. Typhoid bacilli live longer in filtered water than in bouillon; they may even live three months.

Ice may contain great numbers of bacteria; it is well known that freezing does not destroy pathogenic bacteria, such as the typhoid bacillus. Prudden found typhoid bacilli in ice after 100 days, although the number was greatly reduced over that placed in the ice originally.

Disposal of Sewage, is a bacteriological process in many cases; either the sewage may be treated in sand filters or it may be run out on land where over 200,000 gallons may be disposed of on an acre

of land a day. As far as possible nature should be imitated in every way and the breaking up of masses of matter in sewage may be accomplished in the septic tank process in which active oxidization of the matter is accomplished by bacteria. It appears from the observations of many sanitarians that both aerobic and anaerobic bacteria are necessary to finally reduce sewage to the elementary gases and pure water.

In the interior of closed tanks and in the depths of sand filters anaerobic conditions prevail. On beds of coke, and on the surface of sand filters, aerobic conditions obtain. The effluent from a septic tank sewage disposal plant is very often pure water from both chemical and bacteriological standpoints, due to the chemical action of the bacteria.

Bacteriology of the Air.

That the lower layers of the earth's atmosphere contain many bacteria is well known. The air over the sea and over mountain ranges is freer from bacteria than is the air over arable lands and large cities.

When air is still and confined, all bacteria, according to Tyndall, gravitate to the ground, and the air above becomes quite sterile. The atmosphere of sick rooms, hospitals, public conveyances, theatres, etc., contain many bacteria and often pathogenic ones.

The pus cocci, tubercle bacilli, and the organisms causing small-pox, scarlet fever, and measles, all may contaminate the air.

The number of bacteria in a given quantity of air may be accurately measured by means of a Sedgwick-Tucker aerobioscope; this consists of a large cylindrical glass vessel opening at either end into various tubulations. (Fig. 80.) Into one of these granulated sugar may be packed; the ends are then plugged with cotton and the apparatus sterilized. To examine the air, a litre or more is drawn through the sugar and the latter is then shaken into the large cylinder where it is dissolved in melted gelatine culture media. The latter is distributed over the interior of the glass and allowed to harden. All the bacteria that

were in a litre of air having been mixed with gelatine and those that are not strict anaerobes grow in the gelatine and a number of colonies can then be counted.

The dust of dwellings and streets contains most of the bacteria. Dried sputum is ground under foot and swept up in gusts of wind, and the contained bacteria are thus inhaled and do harm. The air coming quietly from the lungs is pure and sterile. Even in active

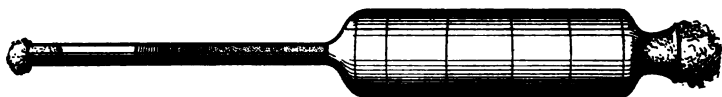


FIG. 80.—Sedgwick-Tucker aerobioscope. (Williams.)

disease processes of the throat this is true. In case the breath comes violently, as in speaking, coughing, and sneezing, the reverse is the case. In general it may be put down as an axiom that disease germs cannot rise from a fluid, such as sewage. Sewer gas, as a rule, is a bearer of some pathogenic bacteria chiefly cocci but in reality it is purer than generally supposed.

Bacteriology of the Soil.

At least two forms of pathogenic bacteria are habitually found in the soil. The tetanus bacillus, it is well known, exists in garden earth, manure, and top soil generally. Dirt getting into wounds is the most frequent cause of tetanus. Drinking water laden with soil has been known to have in it tetanus bacilli, and if used in an unsterile condition in wounds or when a comparatively feeble antiseptic, such as creolin, has been added, it may cause tetanus.

The bacillus of malignant oedema has also been isolated from soil. Streptococci and colon bacilli, too, have been found in garden soil. Typhoid bacilli may contaminate soil, but do not multiply in it. In sandy soil 100,000 bacteria per gram have been found, in garden soil 1,500,000 bacteria per gram, and in sewage polluted

soil 115,000,000 bacteria per gram have been isolated. The first few inches of ordinary soil contain most of the bacteria, after a depth of two metres no bacteria at all are found and the earth is sterile.

Soil may be collected in sterile sharp pointed iron tubes, and diluted with sterile water of given quantity and plates poured from it.

Arable lands may be enriched very much by inoculating them with certain nitrifying bacteria, some of which convert ammonia into nitrous acid, which form in them nitrites; others change nitrites into nitrates (nitrosomonas). Certain of these bacteria are concerned in the assimilation of nitrogen from the atmosphere and adding to the nitrogen content of the soil, thus enriching it. On the roots of some plants, alfalfa, beans, peas, and clover, minute tubercles develop. These little pathogenic growths are caused by the nitrifying bacteria, and add to the nutrition of the plant by adding to it ammonia.

Bacteriology of Cow's Milk.

Theoretically the milk in the interior of the breasts of nursing women and the udders of cows is sterile. So soon as it leaves the nipple it becomes contaminated with bacteria, and by the time it reaches the pail, in the case of cow's milk, it is far from sterile.

Bacteria of the air, and dust from the cattle and bedding, at every movement of the cow, and by the agency of flies, find their way into milk and contaminate it. The number of bacteria that develop in the milk depends upon the number that reach it in the first place, the temperature of the air, and the length of time. Two hundred and thirty-nine different varieties of bacteria have been isolated from milk at different times.

Pathogenic varieties of bacteria that are found in cow's milk include the tubercle bacillus, streptococcus pyogenes, staphylococcus aureus, the colon bacillus, typhoid bacillus, the diphtheria bacillus, and a whole host of bacteria that sour or ferment the milk and render it unwholesome or poisonous for young children.

Cattle may be tuberculous, and the tubercle bacilli may reach the milk in this way. There may be abscesses of the udder and the streptococci from the pus may cause infection in those that use it. Ordinary follicular tonsillitis may be caused in this way.

Bacteria may develop rapidly in milk, which is a good culture medium, until they number many million per cubic centimeter (sometimes 200,000,000).

In good milk the number of bacteria may increase when the temperature is 90° F., from 5,200 originally in the milk immediately after milking, to 654,000 in eight hours.

By exposing milk to a temperature of 165° F. for twenty to thirty minutes (Pasteurization) most of the non-spore bearing bacteria are destroyed, so that the number may be reduced 99.999 percent by this process.

Absolute cleanliness on the part of the milker, the use of sterilized gloves and clothes, the absence of flies, dust, and the immediate disposal of manure, the filtration of the milk after collection, the immediate cooling of it, the uses of sterilized milk cans and bottles, all lessen the bacterial content of milk. It then keeps better, and is a wholesomer and safer food for infants, especially in hot weather.

By drinking water containing typhoid bacilli cows cannot be sources of typhoid infection through the milk. The typhoid bacilli are not transmitted through the bodies and udders of the animals.

TABLE

Representing the Important Morphological and Chemical Characteristics of Bacteria.

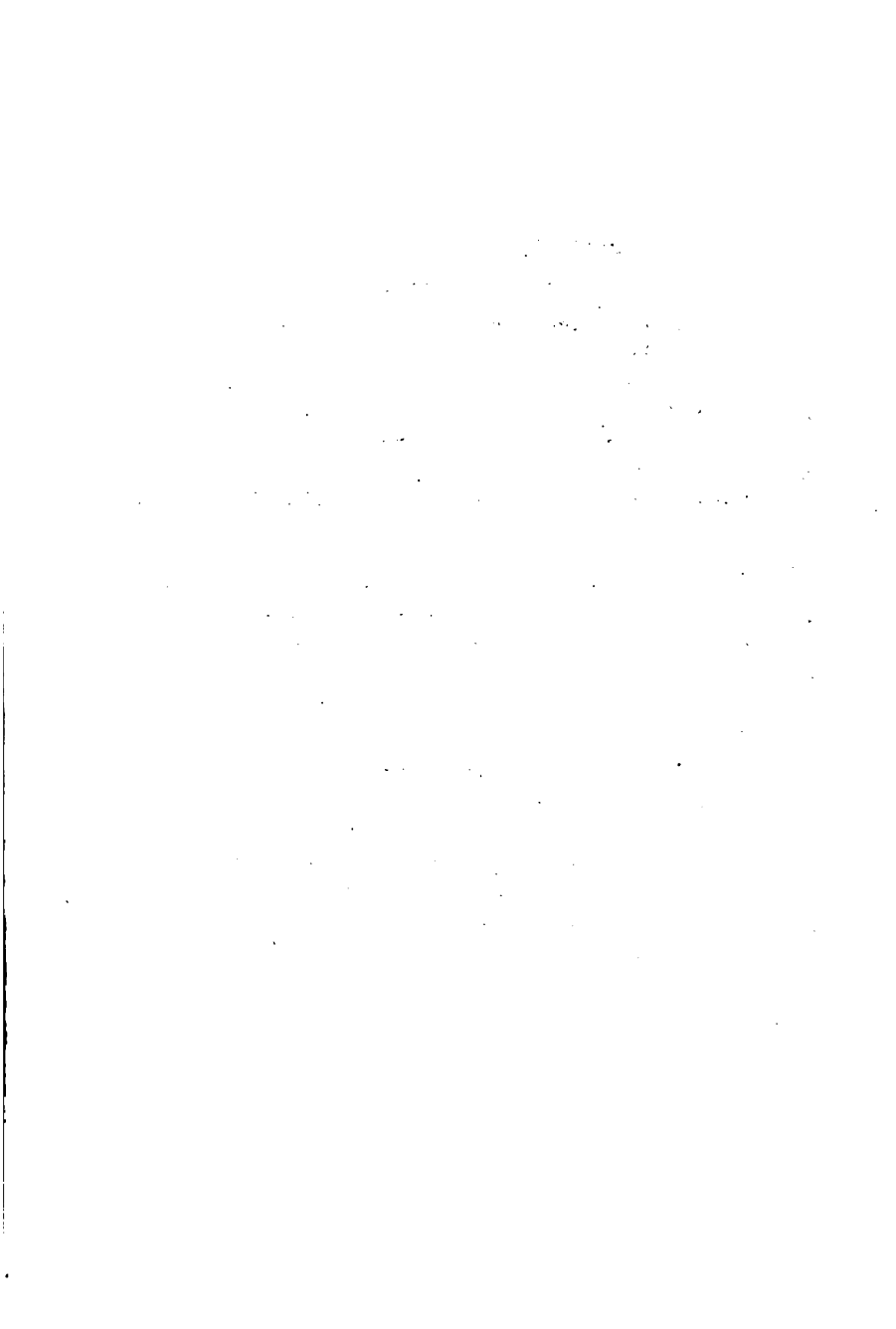
(Arranged From *Lehmann & Neumann.*)

Signs: + indicates presence.
 — indicates absence.
 Δ indicates presence at times and absence at times.

NAME.	FLAGELLA.	GRAM'S STAIN.	AEROBIC AND ANAEROBIC GROWTH.		LIQUIFICATION OF GELATIN.	BOUILLON CULTURE.		MILK CULTURE.		SPORE-FORMATION.	INDOL REACTION.	PRODUCTION OF GAS IN SUGAR-AGAR.
			AEROBIC.	ANAEROBIC.		PELLICULE.	CLOUDINESS.	COAGULATION.	REACTION.			
<i>Streptoc. pyogenes.</i>	—	++	+	+	—	—	—	++	Acid. Acid.	—	—	—
<i>Streptoc. lanceolatus.</i>	—	++	+	+	—	—	—	++	Acid. Acid.	—	—	—
<i>Streptoc. meningitidis.</i>	—	++	+	+	—	—	—	Δ	Faintly alkal. Alkaline.	—	—	—
<i>Mic. tetragenus.</i>	—	++	+	+	—	—	Marked.	+	Faintly alkal. Alkaline.	Very slight.	—	—
<i>Mic. pyogenes a aureus.</i>	—	++	+	+	—	—	—	+	Alkaline.	—	—	—
<i>Mic. pyogenes B citreus.</i>	—	++	+	+	—	—	Slight.	Δ	Faintly acid.	A trace.	—	—
<i>Mic. pyogenes y albus.</i>	—	++	+	+	—	—	—	Δ	Faintly acid.	—	—	—
<i>Mic. gonorrhoea.</i>	—	++	+	+	—	—	—	—	—	—	—	—
<i>Bact. melitensis.</i>	+	—	+	+	—	+	+	—	—	—	—	—
<i>Bact. pestis.</i>	—	—	—	—	—	—	Cloudy at first.	—	—	—	Pronounced.	—

NAME.	FLAGELLA.	GRAM'S STAIN.	AEROBIC AND ANAEROBIC GROWTH.		LIQUEFACTION OF GELATIN.	BOUILLON CULTURE.		MILK CULTURE.		SPORE-FORMATION.	INDOL REACTION.	PRODUCTION OF GAS IN SUGAR-AGAR.
			AEROBIC.	ANAEROBIC.		PELLUCID.	CLOUDINESS.	COAGULATION.	REACTION.			
Bact. influenzae.	—	—	+	—	—	—	Moderate.	—	Acid.	—	Slight.	—
Bact. pneumoniae.	Many.	—	+	+	—	—	Slight.	—	Amphoteric.	—	—	—
Bact. typhi.	A few;	—	+	+	—	—	Moderate.	+	Acid.	—	Pronounced.	—
Bact. coli.	seldom one.	—	+	+	—	—	—	—	—	—	—	+
Bact. prodigiosum.	Many.	—	+	+	+	—	Marked.	+	Acid.	—	Very slight.	—
Bact. dysenteriae.	+	—	+	+	—	—	+	—	+ Acid.	—	+	—
Bact. violaceum.	A few.	—	+	+	—	—	Moderate.	+	Acid.	—	—	—
Bact. enteritidis.	+ 8	—	+	+	—	—	+	+	Acid.	—	—	—
Bact. pyocyaneum.	One.	—	+	+	+	—	Marked.	+	Amphoteric, later alkal.	—	—	—
Bact. Zopfii.	Many.	+	+	+	—	—	Very slight.	—	Faintly alkal.	—	Slight.	—
Bact. vulgare.	Many.	+	+	+	—	—	Moderate.	+	Faintly acid.	—	Very pronounced.	—
Bact. vulgare B mirabilis.	Many.	+	+	+	+	—	Moderate.	—	Faintly alkaline.	—	Faint without nitrite.	—
Bact. erysipelatos suum.	—	+	+	+	—	—	Slight.	△	Amphoteric.	—	—	—
Bac. anthracis.	—	+	+	+	—	—	Very marked.	+	Faintly alkaline.	—	—	—
Bac. mycoides.	—	+	+	+	+	+	Moderate.	+	Alkaline.	—	—	—
Bac. Botulius.	+	+	+	+	+	+	+	—	+ Acid.	+	—	+

NAME.	FLAGELLA.	GRAM'S STAIN.	AEROBIC AND ANAEROBIC GROWTH.		LIQUEFACTION OF GELATIN.	BOUILLON CULTURE.		MILK CULTURE.		SPORE-FORMATION.	INDOL REACTION.	PRODUCTION OF GAS IN SUGAR-AGAR.
			AEROBIC.	ANAEROBIC.		PELLETC.	CLOUDINESS.	COAGULATION.	REACTION.			
Bac. cap. <i>Ærogenes</i> .	—	++	+	+	+	+	Very marked.	+	Acid.	++	—	+
Bac. subtilis.	Many.	++	+	+	+	+	Moderate.	+	Faintly alkaline.	++	—	—
Bac. megatherium.	Many.	++	+	+	+	+	Moderate.	+	Faintly alk.	++	—	—
Bac. vulgarus.	Many.	++	+	+	+	+	Moderate.	Δ	Strongly alk.	++	A trace.	—
Bac. mesentericus.	Many.	++	+	+	+	—	Moderate.	+	Faintly alk.	++	Slight.	+
Bac. tetani.	Many.	++	+	+	+	—	Moderate.	+	Amphoteric.	++	Slight.	+
Bac. Chauvoei.	Many.	++	+	+	+	—	Moderate.	+	Faintly acid.	++	Slight.	+
Bac. oedematis maligni.	Many.	—	+	+	+	+	Moderate.	+	Amphoteric.	—	Pron'd without nitrite.	—
Vibrio cholerae.	One, seldom two.	—	+	+	+	+	Moderate.	+	Acid.	—	Slight.	—
Vibrio Proteus.	One.	—	+	+	+	—	Slight.	+	Faintly alk.	—	A trace.	—
Spir. rubrum.	A bunch.	+	Δ	+	—	—	Moderate.	Δ	Acid.	—	Slight.	—
Corynebact. mallei.	—	+	+	+	—	—	Very slight.	Δ	Amphoteric.	—	Slight.	—
Corynebact. diphtheriæ.	—	+	+	+	—	—	Almost clear.	Δ	Faintly acid.	—	Slight.	—
Coryneb. pseudodiphtheritic.	—	+	+	+	—	—	Clear.	—	Amphoteric.	—	A trace.	—
Corynebact. xerosis.	—	+	+	+	—	+	—	—	—	—	—	—
Mycobact. tuberculosis.	—	+	+	+	—	+	—	—	—	—	—	—
Mycobact. Lepreæ.	—	++	+	+	—	+	—	—	—	—	—	—
Actinomyces bovis.	—	++	+	+	+	—	—	+	Faintly alk.	—	A trace.	—



DESCRIPTION OF PLATE I.

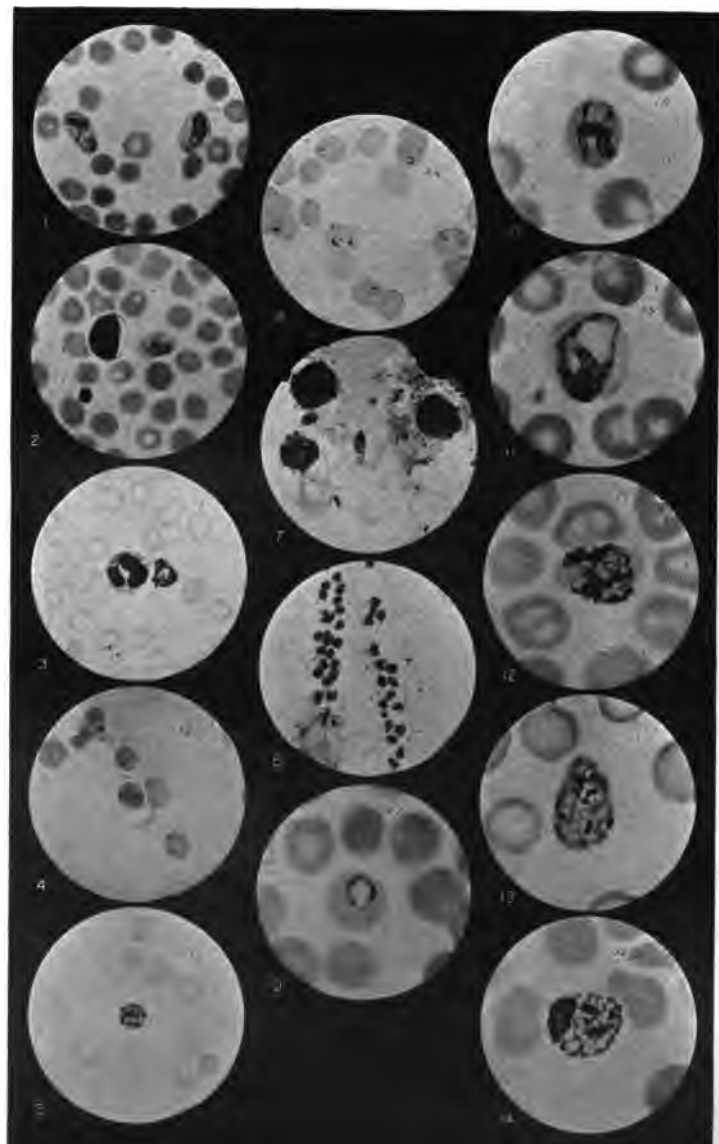
Malarial Parasites.

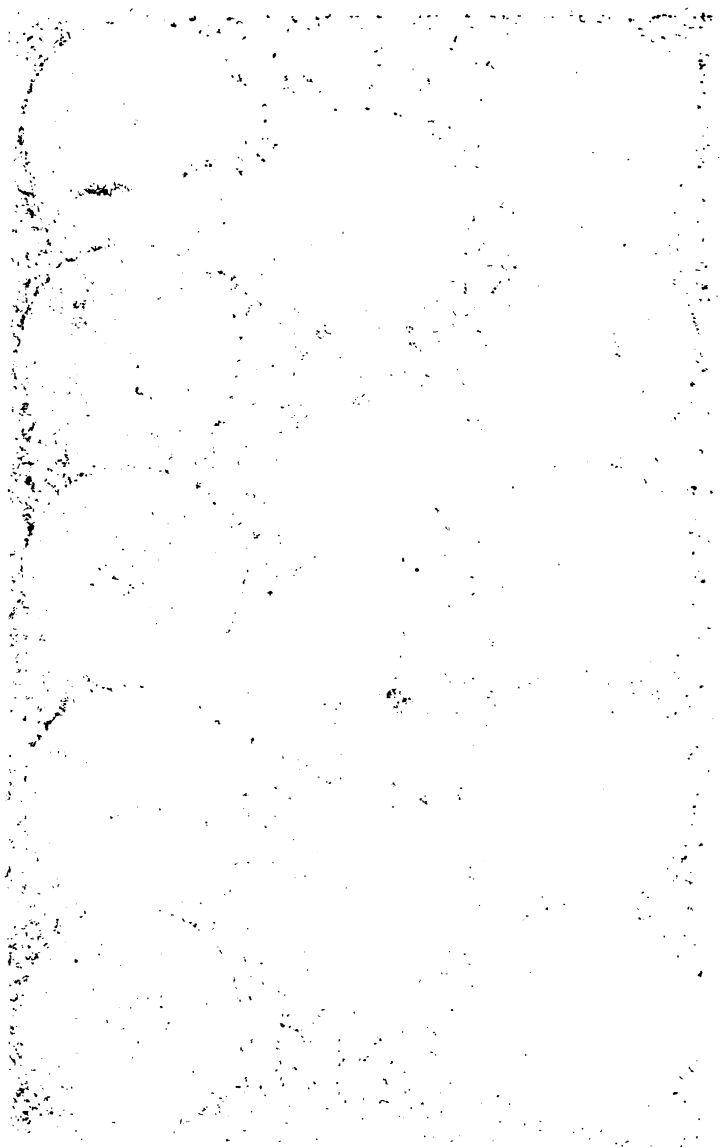
1. Two tertian parasites about thirty-six hours old, attacked blood corpuscles magnified.
2. Tertian parasite about thirty-six hours old; stained by Romanowsky's method. The black granule in the parasite is not pigment but chromatin. Next to it and to the left is a large lymphocyte, and under it the black spot is a blood plate.
3. Tertian parasite, division form nearby is a polynuclear leucocyte.
4. Quartan parasite, ribbon form.
5. Quartan parasite, undergoing division.
6. Tropical fever parasite. (Æstivo-autumnal.) In one blood corpuscle may be seen a smaller, medium, and large tropical fever-ring parasite.
7. Tropical fever parasite. Gametes half moon spherical form. Smear from bone marrow.
8. Tropical fever parasite which is preparing for division heaped up in the blood capillaries of the brain.

Asexual Forms.

9. Smaller tertian ring about twelve hours old.
10. Tertian parasite about thirty-six hours old, so called amœboid form.
11. Tertian parasite still showing ring fever, forty-two hours old.
12. Tertian parasite, two hours before febrile attack. The pigment is beginning to arrange itself in streaks or lines.
13. Tertian parasite further advanced in division. Pigment collected in large quantities.
14. Further advanced in the division. (Tertian parasite.)

PLATE I.



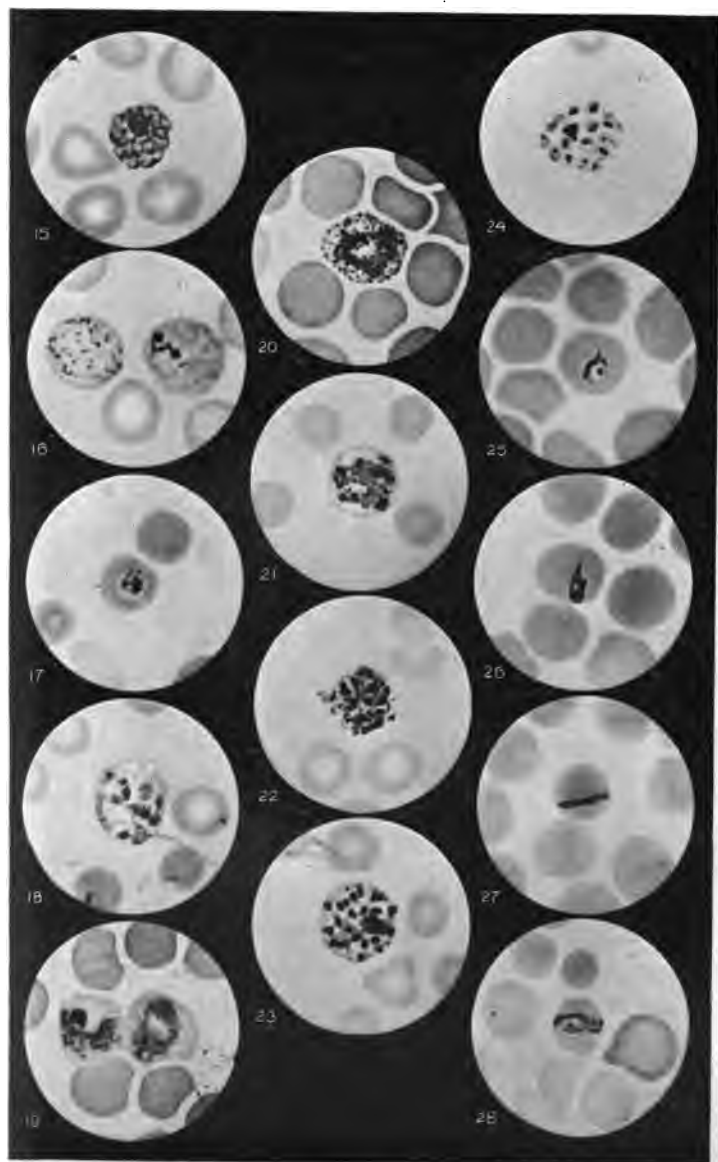


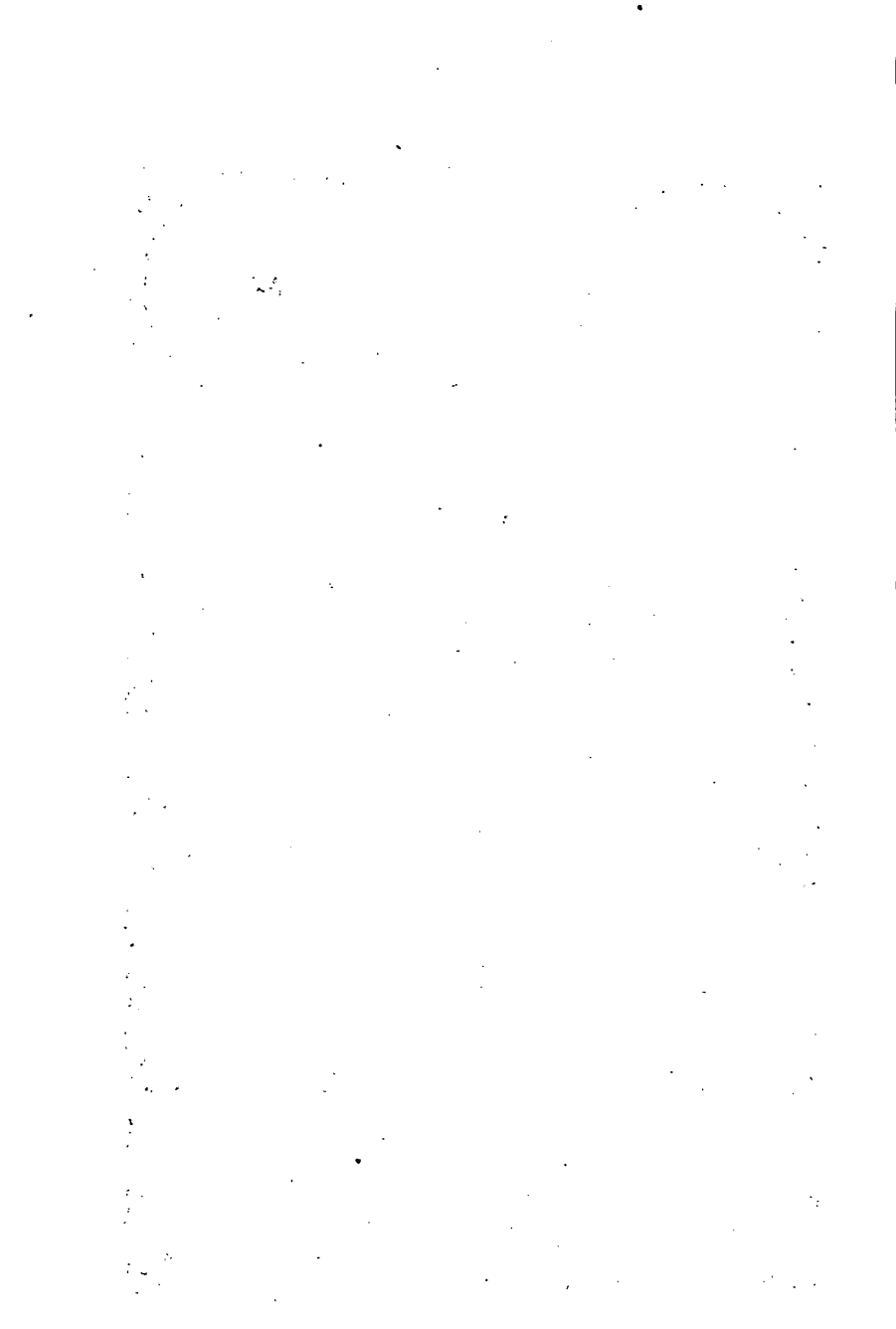
DESCRIPTION OF PLATE II.

Malarial Parasites.

15. Complete division of the parasite. Typical mulberry form.
16. To the left is the completed division form, an almost developed gamete, which is to be recognized by its dispersed pigment.
17. A tertian ring parasite, small size broken up.
18. Three-fold infection with tertian parasite. The oval black granules are the chromatin granules.
19. To the left, tertian parasite with large, sharply demarked, and deeply colored chromatin granules. To the right, tertian parasite. Both thirty-six hours old. Both probably gametes.
20. Tertian parasite thirty-six hours old, ring form.
21. Tertian parasite with beginning chromatin division, with eight chromatin segments.
22. Tertian parasite chromatin division farther advanced with twelve chromatin granules, in part triangular in form.
23. Completed division figure of a tertian parasite. Twenty-two chromatin granules.
24. The young tertian parasites separating themselves from each other. The pigment remains behind in the middle.
25. Quartan ring parasite, which is hard to differentiate from large tropical ring or small tertian ring.
26. Quartan ring lengthening itself.
27. Small quartan ribbon form.
28. The quartan ribbon increases in width. The dark places consist almost entirely of pigment.

PLATE II.







DESCRIPTION OF PLATE III.

Malarial Parasite.

29, 30, 31. The quartan ribbon increases in width. The dark places consist almost entirely of pigment.

32. Beginning division of the quartan parasite and the black spot in the middle is the collected pigment.

33. Quartan ring.

34. Double infection with quartan parasites.

35. Wide quartan band. The fine black stippling in the upper half of the parasite is pigment.

36. Beginning division of the quartan parasite. The chromatin (black fleck) is split into four parts.

37. Division advanced, quartan parasites.

38. Typical division figure of the quartan parasite.

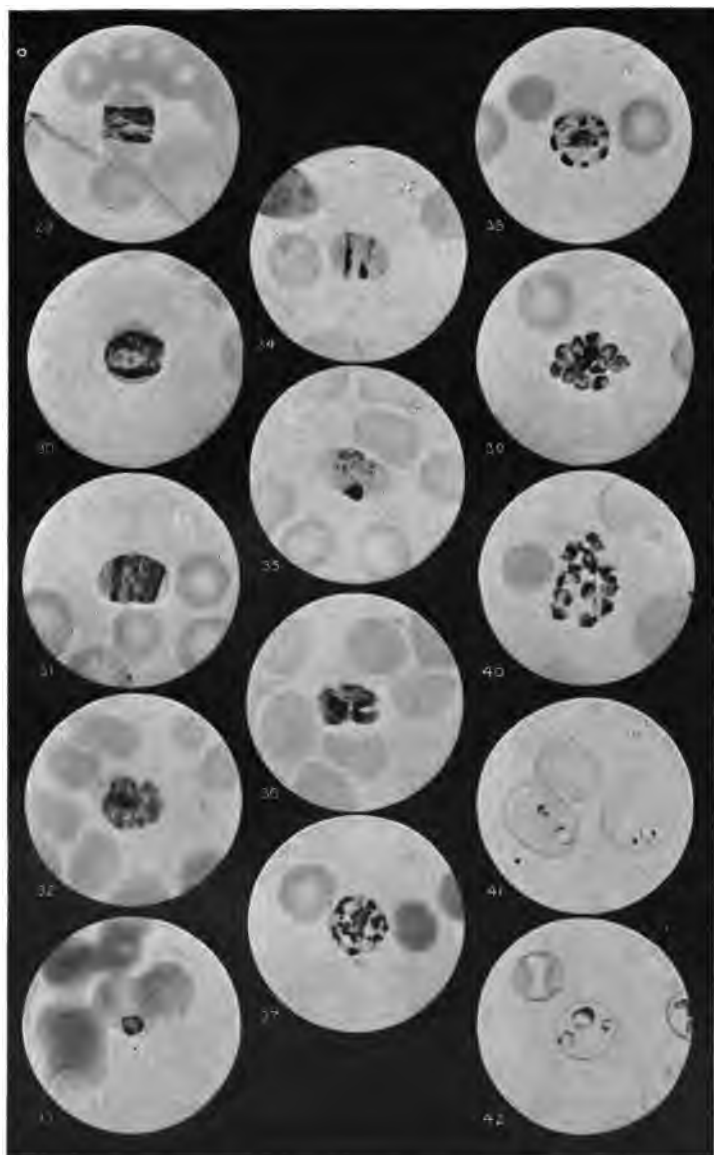
39. Finished division of the quartan parasite. Ten young parasites, pigment in the middle.

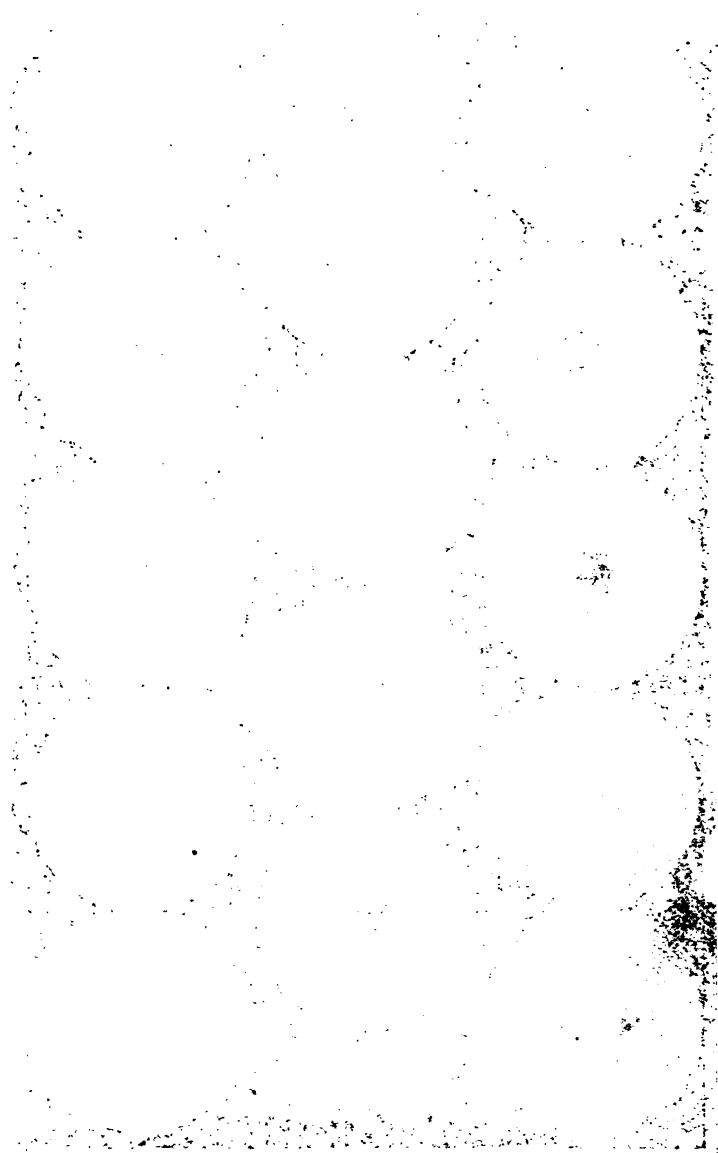
40. Young parasites separated from one another.

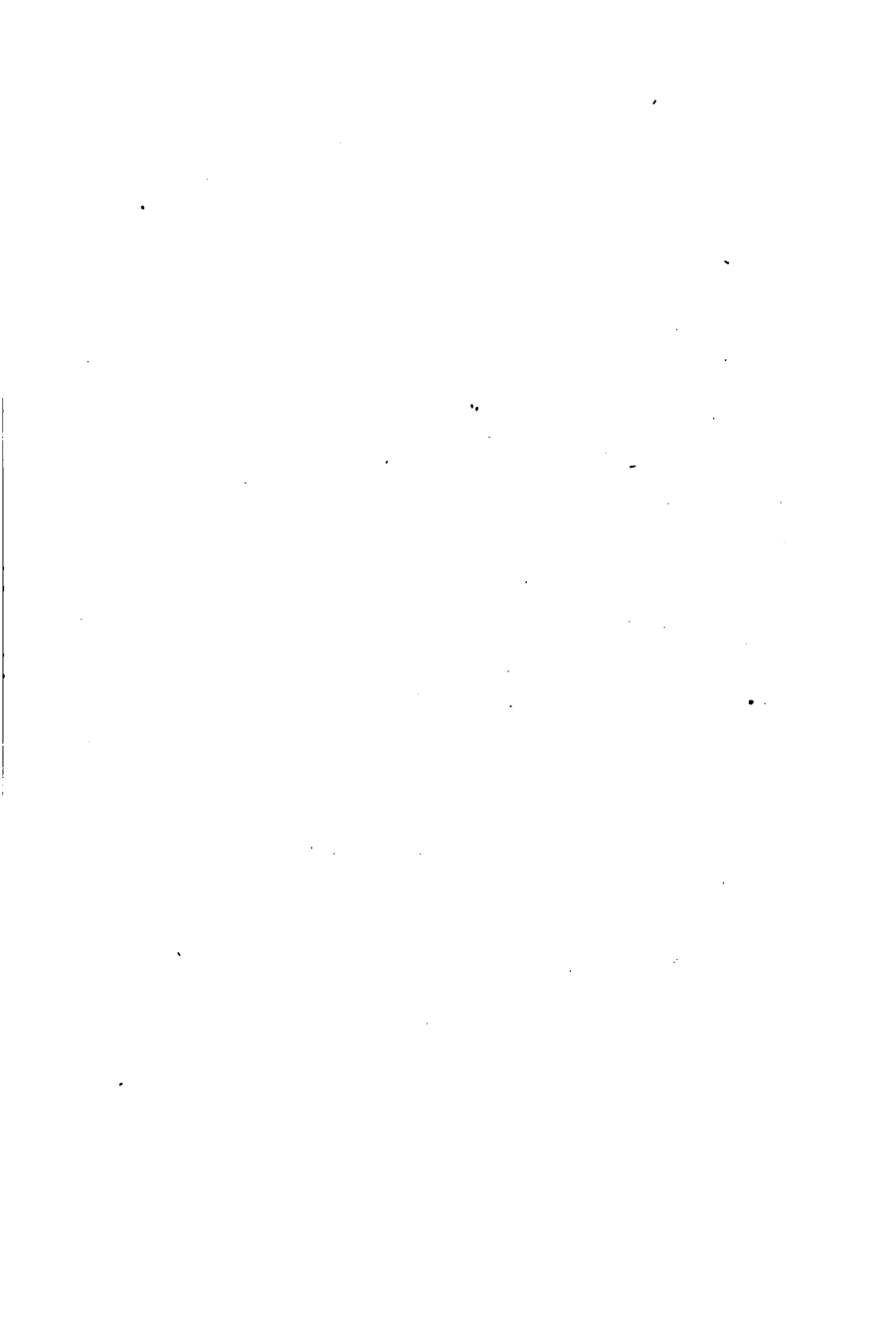
41. Small and medium tropical ring, the latter in a transition stage to a large tropical ring.

42. Small, medium and large tropical ring, together in one corpuscle.

PLATE III.







DESCRIPTION OF PLATE IV.

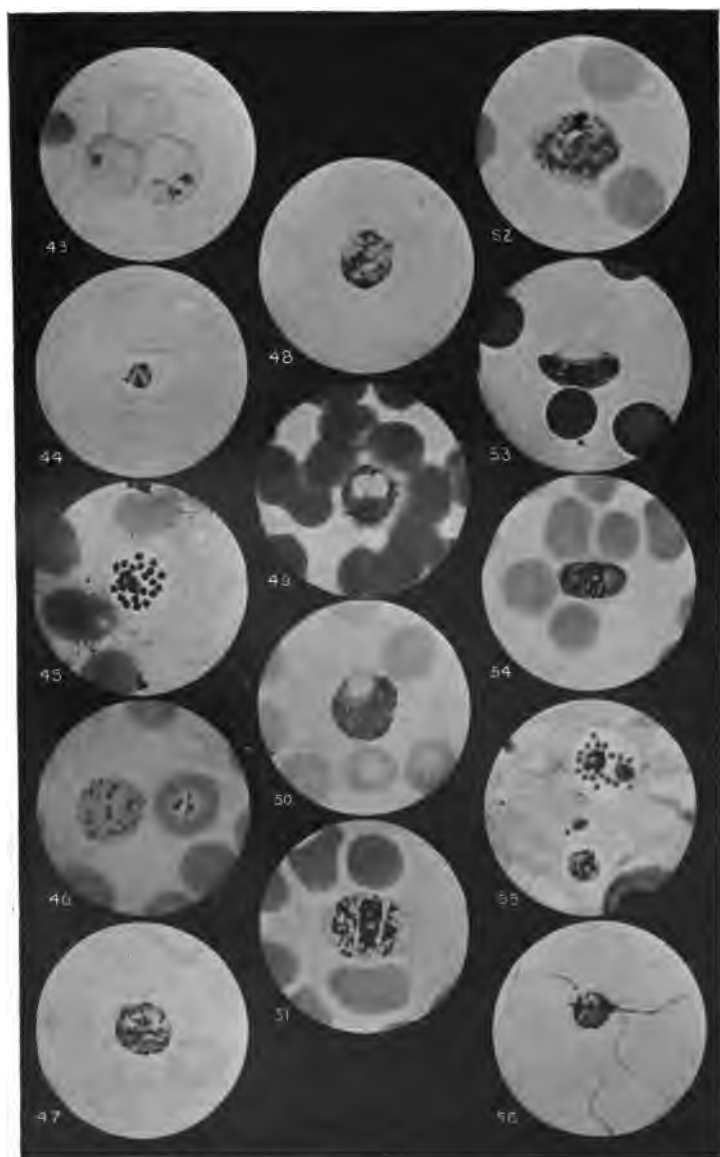
Malarial Parasite.

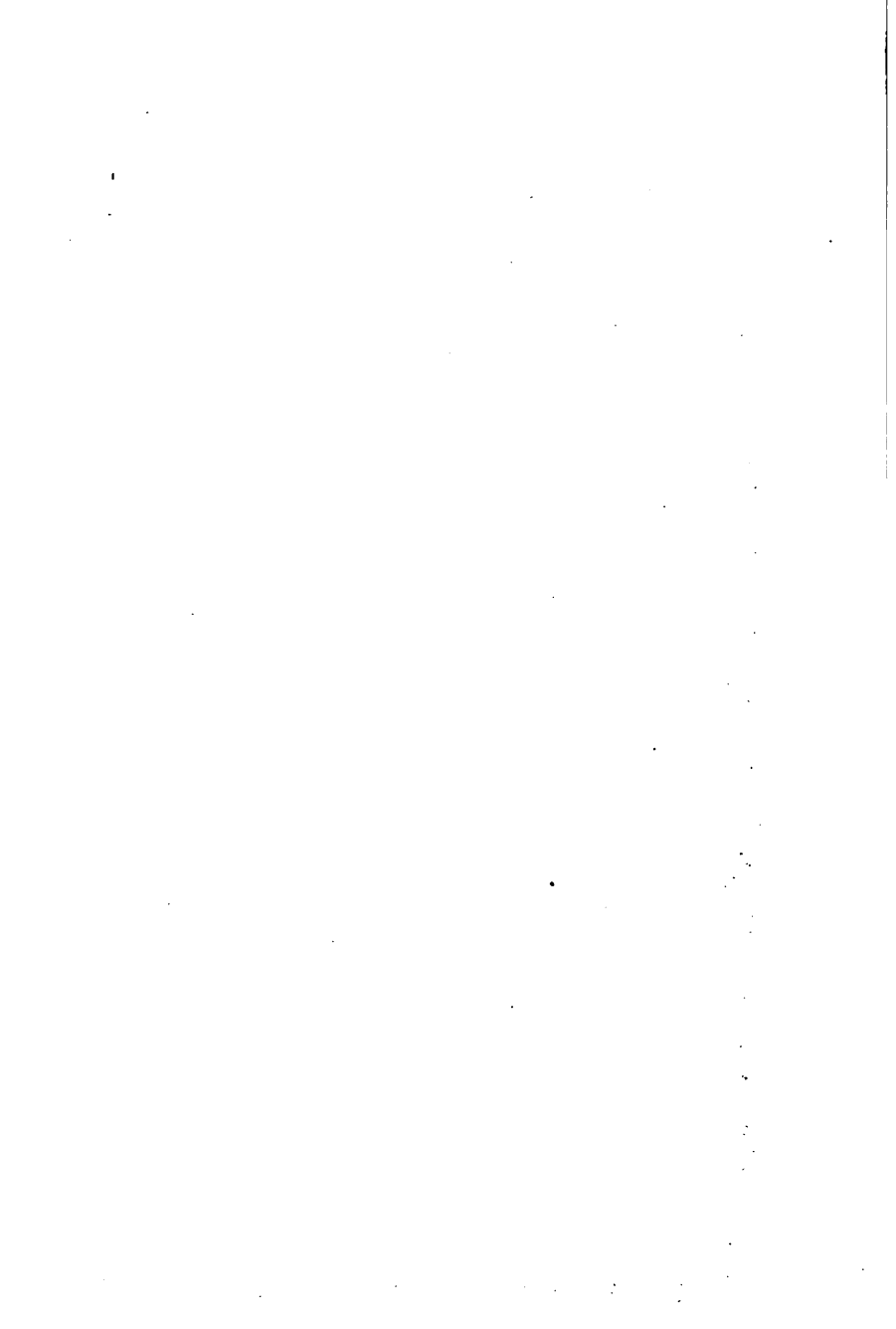
43. To the left a young (spore) tropical parasite. To the right a medium and large tropical parasite.
44. An almost fully developed tropical parasite. The black granules are pigment heaps.
45. Young parasites separated from one another. Broken up division forms twenty-one new parasites.
46. To the left a red blood corpuscle with basophilic, karyochromatophilic granules. Prototype of malarial parasite. On the right a red blood corpuscle with remains of nucleus.

Sexual Forms or Gametes.

47. An earlier quartan gamete (microgametocyte in sphere form), female.
48. An earlier quartan gamete (microgametocyte), male.
49. Tertian gamete, male form (microgametocyte).
50. Tertian gamete, female (microgamete).
51. Tertian gamete (microgametocyte) still within a red blood corpuscle.
52. Microgamete tertian within a red blood corpuscle.
53. Tropical fever. (Æstivo-autumnal) gamete, half moon (crescent) still lying in a red blood corpuscle. In the middle is the pigment. The concave side of the crescent is spanned by the border of the red blood corpuscle.
54. Gamete, tropical fever parasite.
55. Gamete of tropical fever parasite heavily pigmented.
56. Gamete of the tropical fever parasite (flagellated form), microgametocyte sending out microgametes, (flagella or spermatozoon).

PLATE IV.





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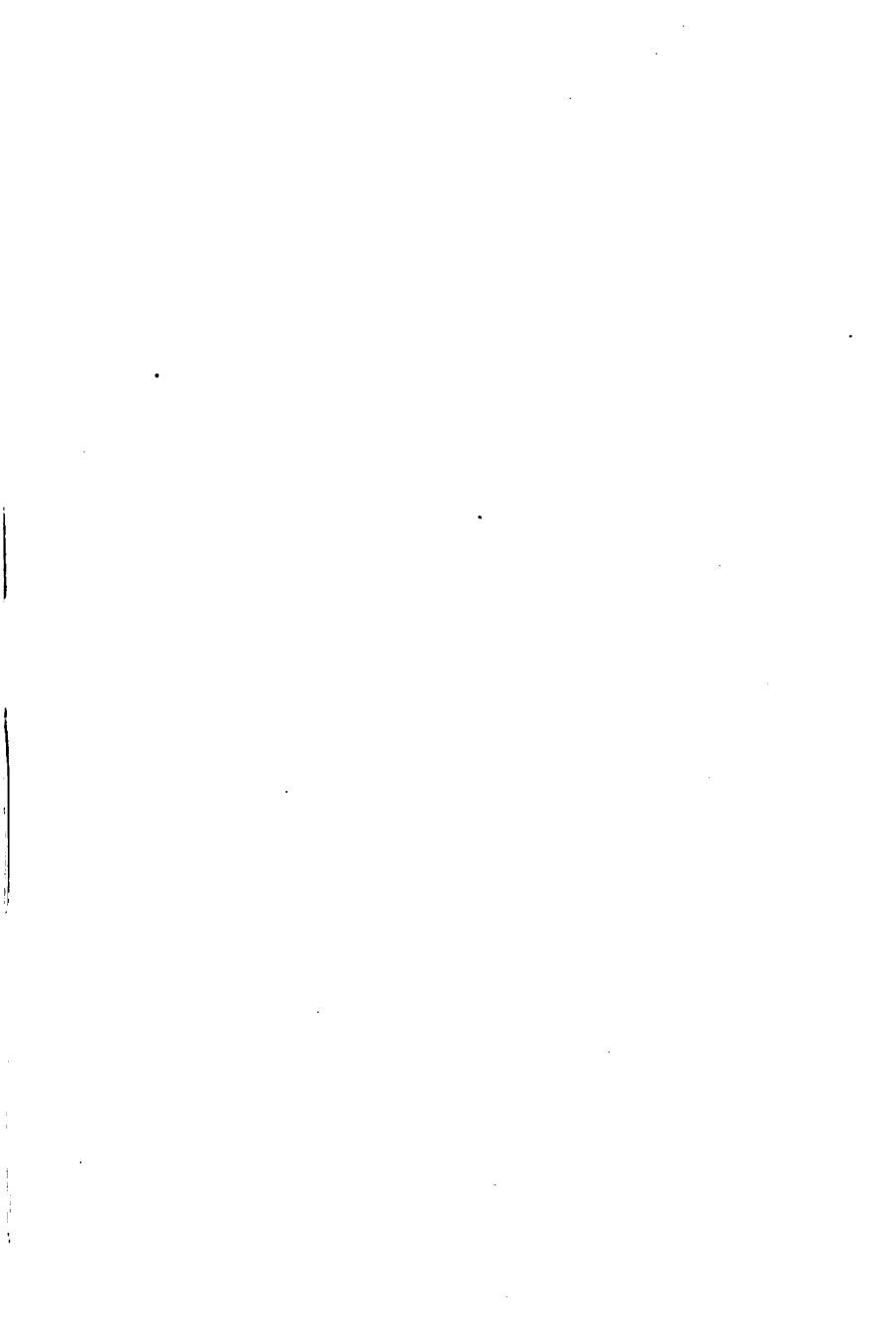
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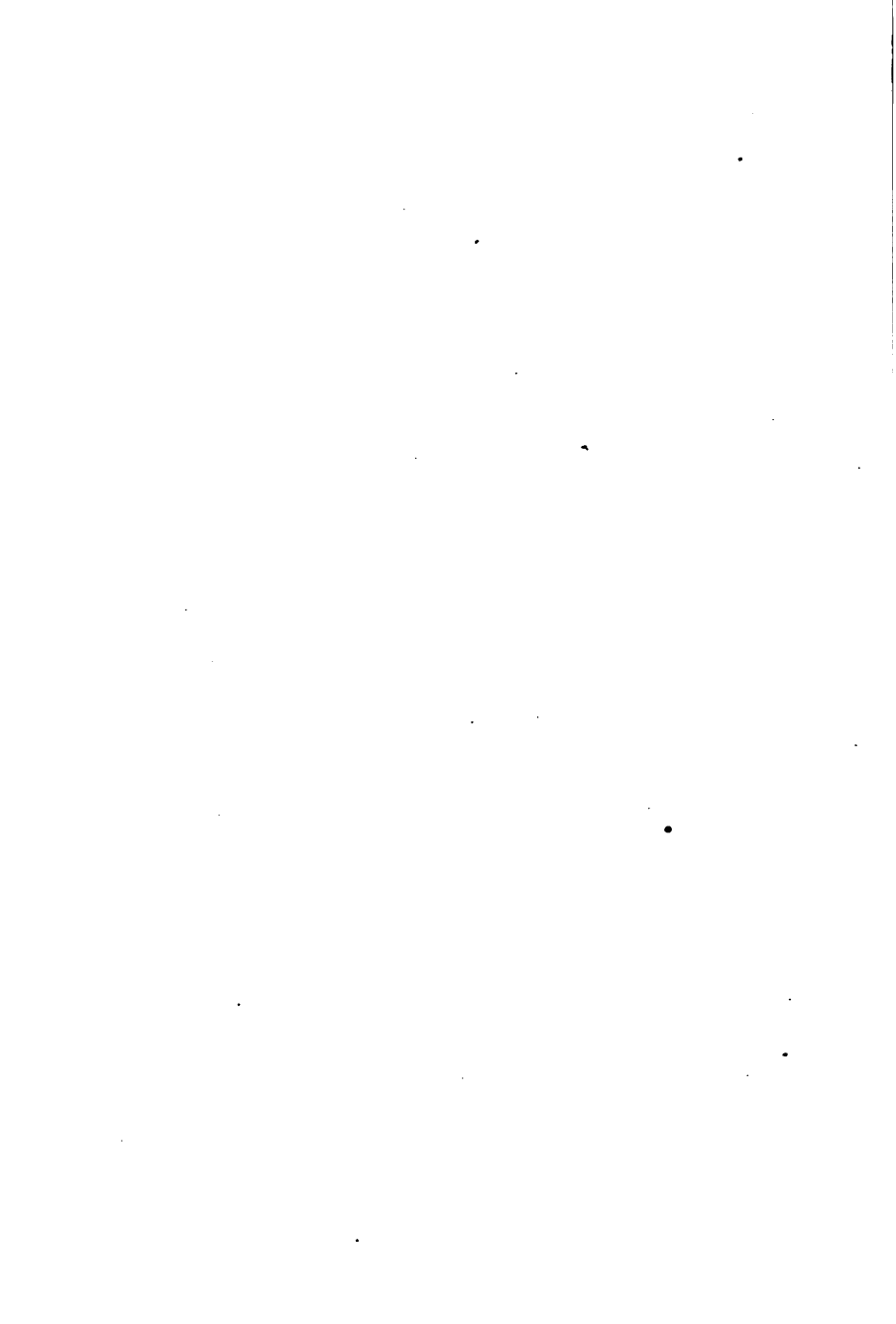
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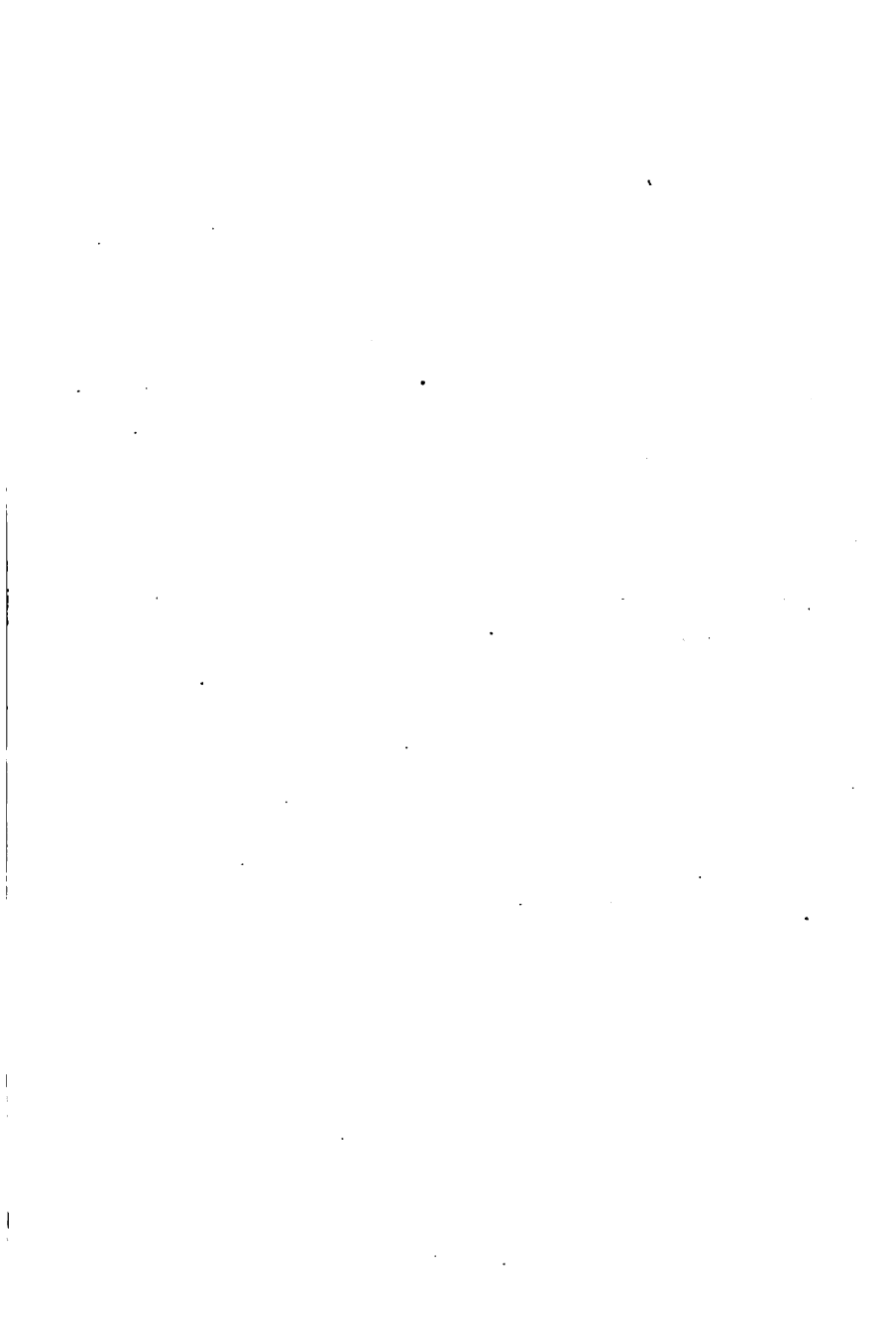
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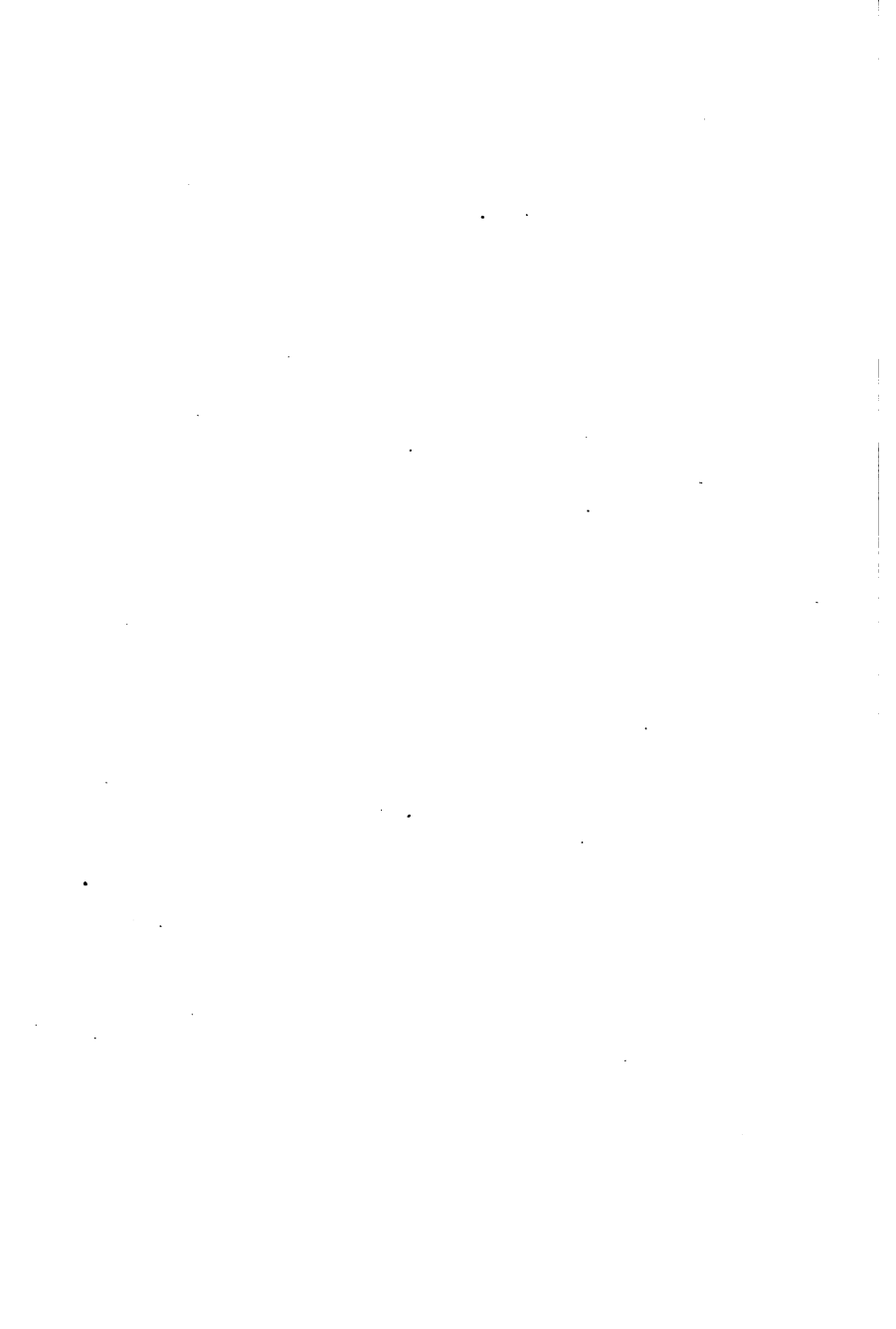
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